HEART RATE VARIABILITY ANALYSIS

AND

ECHOCARDIOGRAPHIC STUDY IN

NORMAL AND HYPOTHYROID (FEMALE) PATIENTS

BEFORE AND AFTER TREATMENT

Dissertation submitted to

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVRSITY

In partial fulfillment of the regulations for the award of the degree of

M.D. (PHYSIOLOGY)

BRANCH- V

INSTITUTE OF PHYSIOLOGY AND EXPERIMENTAL MEDICINE

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CERTIFICATE

This is to certify that the Dissertation entitled "**HEART RATE VARIABILITY ANALYSIS AND ECHOCARDIOGRAPHIC STUDY IN NORMAL AND HYPOTHYROID (FEMALE) PATIENTS BEFORE AND AFTER TREATMENT**" by the candidate Dr. G. Saraswathi in partial fulfillment of the requirements for M.D. PHYSIOLOGY is a bonafide record of the research done by her during the period of study (2013 to 2016) in the Institute of Physiology and Experimental Medicine, Madras Medical College, Chennai- 600003.

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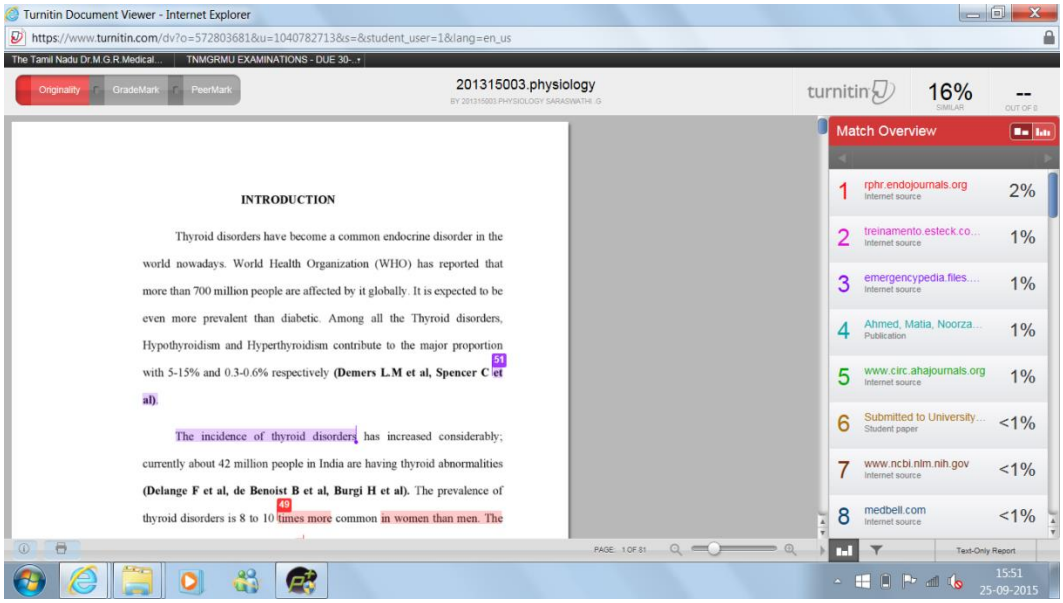
LIST OF ABBREVIATIONS

ANS	Autonomic nervous system
BP	Blood Pressure
DBP	Diastolic Blood Pressure
ECG	Electrocardiogram
FFT	Fast Fourier Transform
HF	Heart rate
HR	Heart rate
HRV	Heart Rate Variability
LF	Low Frequency
NE	Nor Epinephrine
NN 50	Normal to Normal RR interval deviation more than 50 ms
PNS	Parasympathetic Nervous system
PSD	Power Spectral Density
RRI	RR Interval
SBP	Systolic Blood Pressure
SNS	Sympathetic Nervous System
TSH	Thyroid stimulating hormone
VLF	Very Low Frequency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INTRODUCTION
Thyroid disorders have become a common endocrine disorder in the world nowadays. World Health Organization (WHO) has reported that more than 700 million people are affected by it globally. It is expected to be even more prevalent than diabetic. Among all the Thyroid disorders, Hypothyroidism and Hyperthyroidism contribute to the major proportion with 5-15% and 0.3-0.6% respectively (Demers L.M et al, Spencer C et al).
The incidence of thyroid disorders has increased considerably; currently about 42 million people in India are having thyroid abnormalities (Delange F et al, de Benoist B et al, Burgi H et al). The prevalence of thyroid disorders is 8 to 10 times more common in women than men. The
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ABSTRACT

HEART RATE VARIABILITY ANALYSIS AND ECHOCARDIOGRAPHIC STUDY IN NORMAL AND HYPOTHYROID (FEMALES) BEFORE AND AFTER TREATMENT

Degree for which submitted: Doctor of Medicine (MD) in Physiology

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Year : 2014 – 2015

Background:

Hypothyroidism is the second common endocrine disorder next to Diabetes Mellitus. One of the major target organs of hypothyroidism is the cardiovascular system and it is very sensitive to even minimal deviations from normal levels. The cardiovascular manifestation of hypothyroidism includes decreased cardiac contractility,

decreased cardiac output, increase in peripheral vascular resistance. The electrical abnormalities seen in the ECG consists of bradycardia, low voltage complexes, varying degrees of heart block and decreased systolic and diastolic functions of the heart. This is also associated with disturbances in autonomic balance which may lead adverse cardiac events like arrhythmias in these patients. It is also observed that treatment with L thyroxine in these patients will be helpful in preventing these complications.

Aim and objectives:

To assess the Resting Heart Rate Variability and Echocardiography in normal and hypothyroid (female) patients before and after treatment.

Materials and Methods:

The study included thirty newly diagnosed female hypothyroid patients with elevated seum TSH levels and age matched normal healthy individuals as controls. They were assessed with HRV analysis and ECHO before treatment and reassessed after 3 months of treatment with L-thyroxine. After attaining euthyroid levels by measuring the serum TSH levels they reassessed with HRV and ECHO.

Results:

There was autonomic imbalance with increased sympathetic activity and decreased parasympathetic activity in hypothyroid females before treatment as shown by very highly significant ($p<0.000$) changes in frequency domain measures of HRV like increased LF (nu) and decreased HF (nu) and increased LH/HF ratio, decreased time

domain measures. There were also very highly significant ($p<0.000$) changes in ejection fraction and LVID (both end systolic and end diastolic) parameters. After treatment with L-thyroxine the changes were comparable with normal to normal individuals. The elevated serum TSH levels were also shows highly significant ($p<0.001$) after treatment.

Conclusion:

This study shows that replacement therapy with L Thyroxine will play an essential role in preventing the cardio vascular complications of hypothyroidism like autonomic imbalance. As HRV and ECHO are both noninvasive in nature, both of these can be used to assess these patients of hypothyroidism for CVS complications and regular follow up after treatment.

Key words: Hypothyroidism, L-Thyroxine, Heart Rate Variability, Echocardiography

INTRODUCTION

Thyroid disorders have become a common endocrine disorder in the world nowadays. World Health Organization (WHO) has reported that more than 700 million people are affected by it globally. It is expected to be even more prevalent than diabetic. Among all the Thyroid disorders, Hypothyroidism and Hyperthyroidism contribute to the major proportion with 5-15% and 0.3-0.6% respectively **(Demers L.M et al, Spencer C et al)**[1] .

The incidence of thyroid disorders has increased considerably; currently about 42 million people in India are having thyroid abnormalities **(Delange F et al, de Benoist B et al, Burgi H et al)[2].** The prevalence of thyroid disorders is 8 to 10 times more common in women than men. The prevalence of hypothyroidism is higher than hyperthyroidism, higher in females than in males, and increases with age, suggesting that hypothyroid disorders could merit more concern.

It is well documented that diabetes, hypertension, coronary artery diseases which are It has been found that the non – communicable diseases like diabetes, hypertension, Coronary artery heart disease are mostly due to increased stress and sedentary life style. **(Okada K et al)[3]**, and recently it has been postulated that basic pathophysiology behind these disorders is

mainly autonomic imbalance **(Frontoni S. et al, Bracaglia D, et al, Gigli F et al)[4].**

ACTION OF THYROID HORMONE ON CARDIOVASCULAR SYSTEM

Thyroid hormones are the main regulators of the basal metabolism in all major cells of our body and this will have an influence on the autonomic balance **(Buchheit M et al, Simno C et al)[5]**. Generally hypothyroidism is a hypo metabolic state and sympathetic system functions are expected to be decreased in this state **(Jameson JL et al, Anthony P et al, Weetman AS et al)[6].**

Thyroid hormone has multiple effects on the cardiovascular system. These include increased cardiac contractility, increased cardiac output, decreased systemic vascular resistance and electrophysiological and pro-angiogenic effects. The cellular and molecular mechanisms that have been proposed for this include genomic and non – genomic (extra nuclear) actions.

In genomic actions the active form of thyroid hormone, triiodothyronine (T3), enters the nucleus and binds to the specific nuclear receptors (TR alpha and TR beta). The T3 receptor complex then binds to thyroid –hormone response elements (TREs) in the promoter regions of

specific target genes and modifies their expression. These are genomic actions. The more rapid non-genomic actions includes the regulation of membrane ion channels or pumps and may involve the activation of several signaling pathways, including protein kinase C (PKC), mitogen activated protein kinases (MAPKs) 6, 7 and Akt8.

The heart and the peripheral circulation are the most commonly affected system by the thyroid dysfunction **(Ievleva GI et al, Teslia EF et al, Buganov AA et al)**[7] and many of the signs and symptoms of seen in hypothyroidism could be because of the reduced activity of thyroid hormone on CVS **(Galetta F et al, Franzoni F et al, Fallahi P et al)**[8].

It has long been recognized that decreased thyroid hormone activity may produce cardiovascular abnormalities like bradycardia, decreased cardiac output, diastolic dysfunction, mild diastolic hypertension, increased peripheral vascular resistance, dilated cardiomyopathy and increased incidence of coronary atherosclerosis which are associated with impaired sympatho-vagal balance of the heart.

It has been observed that in patients with defective thyroid function there may a reduced autonomic modulation of cardiac activities **(Galetta F et al, Franzoni F et al, Fallahi P et al)**[8]. This has also been reported that in hypothyroid individuals may have autonomic nervous system disturbance

with a increased level of vagal activity which may be corrected with thyroxine treatment **(Xing H et al, Shen Y et al, Chen H et al)**[9].

Heart diseases are more alarming today due to their life threatening complications. Health awareness among the public also increasing nowadays and they are ready to undergo available required investigations to detect their diseases as early as possible.

From a large number of studies we have found out the significant relationship between the autonomic nervous system and cardiovascular morbidity including sudden cardiac death **(Galetta F et al, Franzoni F et al, Fallahi P et al)**[8]. There were also studies to show the association of lethal arrhythmias with signs of either increased sympathetic or decreased parasympathetic activity **(Barczynski M et al, Tabor S et al, Thor P et al**)[10]. This has encouraged for the development of quantitative markers of autonomic activity. One such that is the Heart rate variability analysis which was used to evaluate the sympathovagal balance in newly diagnosed hypothyroid female individuals in this study.

HEART RATE VARIABILITY ANALYSIS

Heart rate variability analysis is an analysis of beat to beat variations in heart rate, is an important and widely used non- invasive method to evaluate the integrity and autonomic function of heart. **Levy MN et al[11],**

says that heart rate is due to intrinsic activity of Sino atrial node (pacemaker of heart) and the modulating influences of autonomic nervous system.

The intrinsic firing rate of un - innervated human SA node is 100 beats per minute **(Ganong WF et al)[12].** If it is innervated, the sympathetic stimulation will increase the heart rate, while parasympathetic stimulation through the vagus nerve will decrease the heart rate. Normally there is predominance of vagal tone over sympathetic tone which keeps the resting heart rate below 100 per minute.

Heart rate in an individual is measured by counting the number of heart beats per minute. Even in the resting state the duration of cardiac cycle of all heart beats occurring in one minute, is not at all same. There is beat to beat variability of RR intervals in milliseconds. This beat to beat variation is known as HEART RATE VARIABILITY. The RR interval is defined as the interval of time between the two consecutive heartbeats and it is measured in millisecond (ms). It can be measured from Electrocardiography. The autonomic balance between sympathetic and parasympathetic nervous activities on the heart can be determined by analysing the variations of this interval.

The fact that autonomic innervation of SA node produces variability in heart rate has been proved by doing tests that stimulates these

innervating nerves. The heart rate is being constantly influenced by external and internal stimuli and regulated by the autonomic nervous system. This variation of the heart rate from beat to beat is known as the heart rate variability (HRV). Heart rate variability reflects the amount of changes in the length of the intervals. This can be assessed by a group of tests called as cardiac autonomic function tests which evaluate both parasympathetic and sympathetic functions. These tests asses both the resting and reflex response in heart rate variability to different challenges. All these tests are assigned as recommended by (Task Force) which includes the Resting Heart Rate variability.

The Resting Heart Rate variability is a noninvasive, easy to perform and reproduce and are both sensitive as well as specific **(Genovelyand et al, Pfeifer et al)[13].** An increased HRV indicates the heart's ability to adapt quickly and more flexibly to external and internal influences due to an optimally balanced interplay between the sympathetic and parasympathetic nervous systems. A decreased HRV indicates a reduced capacity for adaptation and may suggest impaired autonomic function and serious health impairment.

The clinical features of hypothyroidism are suggestive of sympathovagal imbalance and altered autonomic functions can be improved

after attaining euthyroid state with L thyroxine replacement therapy **(Vijaya Lakshmi et al, N Vaney et al,)**[14].

Thus our present study proceeds with testing Resting heart rate variability on newly diagnosed hypothyroid females and its comparison after replacement therapy with L- thyroxine

Hypothyroidism not only produces sympathovagal imbalance but has also been found to be associated structural and functional changes in the cardiovascular system and hemodynamics. Some of the histopathological changes seen in the hypothyroid cardiomyopathy patients include myofibrillar swelling, loss of stratum and interstitial fibrosis leading on to pale, dilated, flabby heart with pericardial effusion. These changes may produce slowed diastolic relaxation and filling, causing diastolic dysfunction. These alterations may lead to decreased systolic function in severe disease **(Cancuso L et al, Lo Bartolo G et al**)[15].

These structural and functional changes in hypothyroidism can be assessed by Echocardiography which is a very good noninvasive method. The various parameters assessed in Echocardiography were myocardial wall thickness, cardiac chamber size, left ventricular ejection fraction, stroke volume, cardiac output. These changes could be improved after treatment with L thyroxine replacement therapy **(Jagdish et al, H Singh et al)**[16]**.**

Therefore in our study, the sympathovagal balance was assessed by doing the Resting heart rate variability analysis and assessment of cardiac functional status by doing ECHO in the newly diagnosed hypothyroid female individuals and compared with normal controls before and after treatment with L-thyroxine.

REVIEW

OF

LITERATURE

REVIEW OF LITERATURE

THYROID GLAND - A BRIEF INTRODUCTION

Thyroid gland is the largest endocrine gland which secretes the hormones Thyroxine (T4), Triiodothyronine (T3), and Calcitonin. It is one of the first endocrine gland of the body to develop, on approximately the 24th day of gestation. It is highly vascularized with blood flow at a rate of (4-6ml/gram/ minute). The gland is made up of closely packed spherical follicles or acini. The follicular epithelium may be cuboidal or columnar in type depending upon the activity of gland. Each follicle is filled with proteinaceous material called colloid. The other hormone Calcitonin is secreted by the para follicular C cells which is concerned with Calcium homeostasis.

The normal human thyroid gland secretes about 80µg (103nmol) of T_4, 4µg (7 nmol) of T_3, 2µg (3.5 nmol) of RT_3 per day **(Ganong's Text book of Physiology Page no.342 24th edition)[12].** Thyroid hormone synthesis is regulated by negative feedback mechanism operating through pituitary TSH levels which in turn is influenced by hypothalamic TRH levels.

Events in Thyroid History

1915 - Edward Kendall, isolated thyroxine in crystalline form

1952 - Rosalind Pitt Rivers and Jack Grass discovered triiodothyronine.

1961 - Calcitonin was discovered

1988 - gene for beta subunit of TSH was cloned

1989 - gene for TSH receptor was cloned

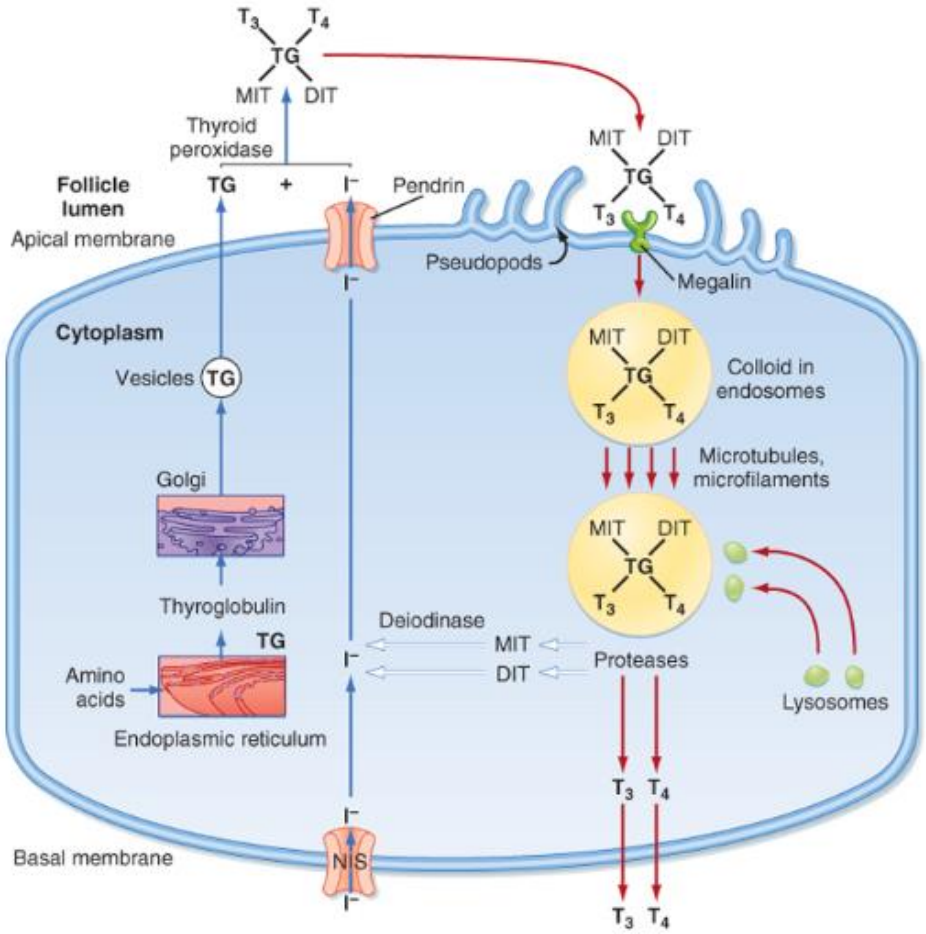


Figure 1: Stages of thyroid hormone synthesis

Physiologic actions of thyroid hormones

Thyroid hormones have widespread actions in the body, secondary to stimulation of oxygen consumption

- They have chronotropic, ionotropic effect in the heart by increasing the number of ß adrenergic receptors, enhanced responses to circulating catechol amines, increased proportion of α myosin heavy chain with (higher ATPase activity)
- In adipose tissue- it stimulates lipolysis
- In Muscles it causes increased protein breakdown
- In Bones it promotes normal growth and skeletal development
- In Gut it increases the rate of carbohydrate absorption
- Others include calorigenic effect, increased oxygen consumption by metabolically active tissues (except testes, uterus, lymph nodes, spleen, anterior pituitary) and increases metabolic rate.

ACTION OF THYROID HORMONE ON CARDIAC MYOCYTE.

Thyroid hormones exert its effect on the heart through genomic and non-genomic actions. The genomic actions of triiodothyronine (T3) are mediated by high-affinity nuclear receptors

that directly regulate gene expression. But the non-genomic effects are rapid and are unaffected by inhibitors of transcription and protein synthesis.

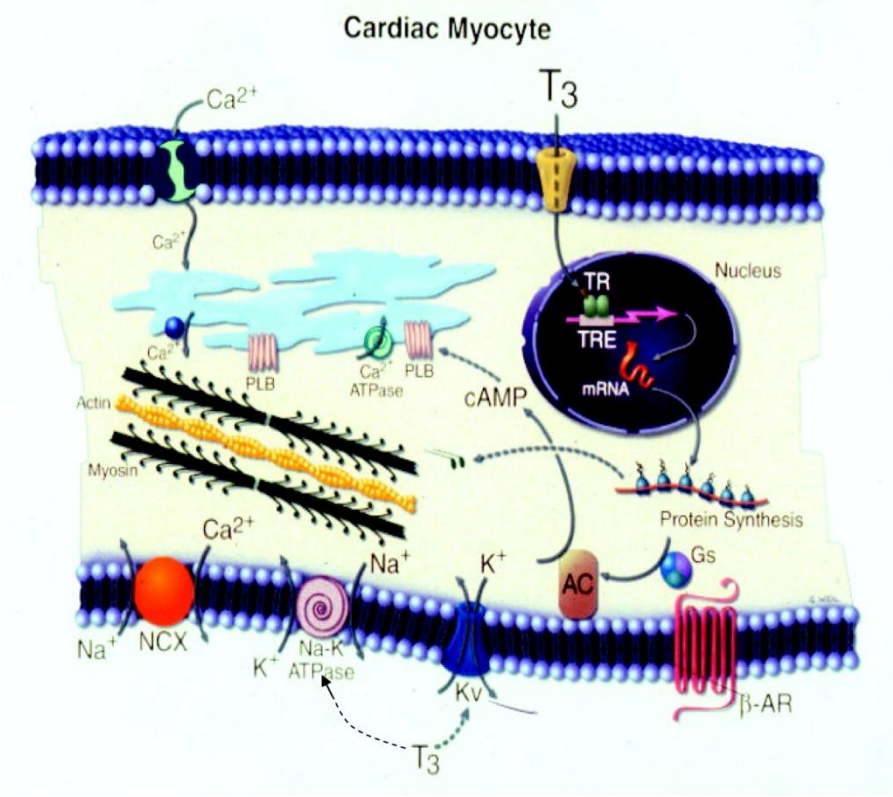


Figure 2: Mechanism of action of thyroid hormone on cardiac myocyte**.** AC indicates adenylyl cyclase; -AR, adrenergic receptor; Gs, guanine nucleotide binding protein; Kv, voltage-gated potassium channels; NCX, sodium calcium exchanger; and PLB, phospholamban.

GENOMIC ACTIONS:

The genomic actions of the thyroid hormone are brought by either activation of transcription or repression of specific genes that will encode the structural and functional proteins **(Dillmann, et al, 1990)[17].** After the entry of active thyroid hormone T_3 directly into the cardiac myocyte, it combines with receptors in the nucleus **(Everts et al, 1996)[18].** Then it

interacts with nuclear receptor α1 (activator) or nuclear receptor α2 (repressor). On binding with these receptors, along with recruited cofactors, the thyroid hormone-receptor complex will bind or release some specific sequences of DNA which are known as thyroid responsive elements. This in turn will act as cis- or trans-regulators that can alter the r transcription speed of specific target genes **(Brent et al, 1994)[19].**

Positively regulated	Negatively regulated
α myosin heavy chain	ß myosin heavy chain
Sarcoplasmic reticulum Ca^{2+}-ATPase	Phospholamban
Na^{+}-K^{+}-ATPase	Adenylyl cyclase catalytic subunits
$ß_1$-adrenergic receptor	Thyroid hormone receptor α_1
ANP	Na^{+}-Ca^{2} exchanger
Voltage gated K^{+} channels	

Table shows Effect of thyroid hormone on cardiac gene expression:

The proteins that are modulated in the transcriptional level includes the myosin heavy chain **(Morkin, 1993 et al[20]; Ojamaa et al, 1996[21])** and calcium activated ATPase that regulates the intracellular calcium and phospholamban **(Dillmann, 1990; Kiss et al, 1994)[17].**

Previous studies done in vitro and in vivo showed that thyroid hormone increase the expression of the α myosin heavy chain in cardiac myocytes, while it decreases the ß isoform **(Morkin, 1993 et al[20]; Ojamaa et al, 1996[21]).** In humans,The ß myosin heavy chain is more pronounced in humans than α isoform **(Magner J, Clark W, Allenby P** 1988**)[22]** and the ratio between the two will be modified slightly by thyroid hormone **(Landenson et al, 1992)[23].**

Abnormalities of cardiac function in patients with thyroid dysfunction directly reflect the effects thyroid hormone on calcium activated ATPase and phospholamban. These are involved primarily in the regulation of systo diastolic calcium concentrations in cardiac myocytes. During diastole the rate of calcium reuptake into the lumen of sarcoplasmic reticulum is determined by calcium activated ATPase. This controls the velocity of myocardial relaxation after contraction **(Dillmann, 1990; Kiss et al 1994)[17].**

The level of phospholamban expression will influence the activity of sarcoplasmic reticulum calcium-activated ATPase (**Kiss et al, 1994)[24].** It has been well demonstrated that the expression of sarcoplasmic reticulum calcium activated ATPase is up regulated and expression of phospholamban is down regulated **(Dillmann, 1990: Kiss et al 1994)[17].** The myocardial contractility is increased by calcium reuptake during diastole.

The expression of other ion channels such as Na-K ATPase, Na+/Ca++ exchanger, voltage –gated K+ channels will help in coordinated electrochemical and mechanical responses of the myocardium **(Gick et al, 1990: Ojamaa et al, 1999)[25].**

The two myosin heavy chains (MHC) present in the heart are alpha-MHC and beta-MHC, which are encoded by two homologous genes located on the short arm of chromosome 17. Each myosin molecule will have two heavy chains and two pairs of light chains. The beta-MHC has less ATPase activity than the alpha-MHC. Atria has alpha MHC predominantly and on treatment with thyroxine its level will be increased thus causing increase in the rate of cardiac contraction. But in hypothyroidism, the expression of the alpha-MHC gene is depressed and beta-MHC is enhanced.

NON-GENOMIC EFFECTS:

This non-genomic actions of the thyroid hormone is more rapid than the genomic actions. Acute phosphorylation of phospholamban occurs which diminishes the inhibition of phospholamban on calcium -activated ATPase in the sarcoplasmic reticulum. This involves the activation of intracellular kinase pathways. These are the pathways involved in signal transduction of the adrenergic stimulus **(Ojamaa et al, 2002)[21].** In this way similar in functions of catechol amines and thyroid hormones on the cardiovascular system could be explained **(Levyandklein,1990)[26].**

EFFECT OF THYROID HORMONE ON CARDIOVASCULAR HEMODYNAMICS

Thyroid hormone reduces peripheral vascular resistance by causing relaxation in vascular smooth muscle cells **(Klemperer et al, 1995: Ojamaa et al, 1996; Park et al, 1997)[27].** Thyroid hormones can cause extra heat production which leads to a slight rise in body temperature, and in turn activate the heat-dissipating mechanisms. Peripheral resistance decreased due to cutaneous vasodilation and this increases renal Na+ and water absorption, expanding blood volume. Cardiac output is increased by direct, as well as due to action of catecholamine on the heart. The pulse pressure and heart rate are increased and circulation time is shortened.

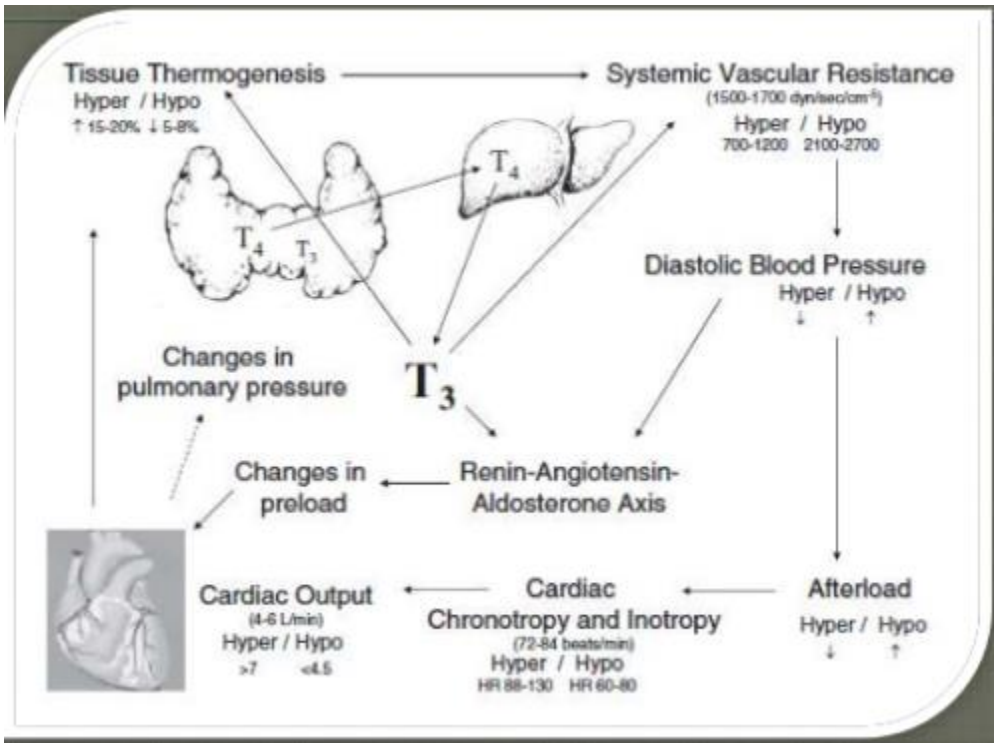


Figure3. Effect of thyroid hormone on cardiovascular hemodynamics

RELATION BETWEEN THYROID HORMONES AND CATECHOL AMINES:

The actions of thyroid hormones and catechol amines secreted by adrenal medulla are closely interrelated. Adrenaline or Epinephrine also increases the rate of metabolism, nervous system excitation and cardiovascular actions similar to thyroid hormones. But all these actions are very rapid. Nor adrenaline or nor epinephrine is also similar in action **(Goodman HM, 2000)**[28]. In hypothyroidism there is impaired cyclic AMP response to epinephrine which suggests decreased responsiveness to catecholamine. This reduced responsiveness is associated with all steps of catecholamine signaling, including receptor and post receptor actions **(Larson PR, et al. 2003)**[29].

T_3 may enhance sympathetic nervous system activity by increasing the number of β-adrenergic receptors in heart muscle and the generation of intracellular second messengers, such as cAMP **(Berne and Levy 6th edition)**[30]**.** Presumably, the functional synergism observed between catechol amines and thyroid hormones, particularly in pathological settings, arises from their overlapping biological functions as well as the ability of thyroid hormones to increase expression of catecholamine receptors and the signaling effectors to which they are linked **(Ganong et al)**[12]**.**

THYROID FUNCTION TESTS

Laboratory tests can be divided into five major categories: (1) those that assess the state of the hypothalamic-pituitary-thyroid axis; (2) estimates of the T_3 or T_4 concentrations in the serum; (3) tests that reflect the impact of thyroid hormone on tissues; (4) tests for the presence of autoimmune thyroid disease; and (5) tests that provide information about thyroidal iodine metabolism.

Thyroid gland functions can be assessed nowadays by highly sensitive and specific hormonal assays. Based on the classic "feedback" loop mechanism whereby levothyroxine (T_4) and triiodothyronine(T_3) regulate pituitary synthesis and release of thyrotropin a thyroid-stimulating hormone (TSH), it is possible with a highly sensitive TSH assay to establish a diagnosis of thyroid disease in essentially every case **(Demers LM et al, Levey GS, Klien I et al)**. In patients with overt hypothyroidism, the lack of T_4 feedback leads to TSH levels -20 mIU/ml, whereas in milder or subclinical hypothyroidism the TSH levels are between 3 and 20 mIU/ml with normal T_4 and T_3 levels **(Demers LM et al, Surks et al, Oritz I et al).** Thus the TSH test is the appropriate initial test to screen for thyroid dysfunction in a variety of clinical situations known to be affected by thyroid disease as well as to confirm a suspected diagnosis and follow the response to treatment.

Hormone	Normal levels
TSH	0.4-4.2mIU/ml
Free T_3	0.2-0.5ng/dl
Free T_4	0.7-2.5ng/dl
Total T_3	70-190 ng/dl
Total T_4	5-11µg/dl

Table shows normal levels of thyroid hormones. (**William's text book of Endocrinology)**[31]**.**

HYPOTHYROIDISM

Hypothyroidism is the second commonest endocrine disorder next to Diabetes mellitus**.** Hypothyroidism is a clinical state due to reduced thyroid hormone levels in circulation. It can be classified as primary hypothyroidism due to transient or progressive impairment of thyroid hormone biosynthesis. Central or secondary hypothyroidism is due to insufficient stimulation of the normal gland due to disease of hypothalamus, or pituitary, or due to defects in TSH molecule. 99% of hypothyroidism is due to primary causes and less than 1% due to secondary causes or TSH defects (William's Text book of Endocrinology).

HYPOTHYROIDISM - CLINICAL FEATURES:

- Easy fatigability,
- Extreme somnolence

- Muscular weakness
- Delayed ankle jerk
- Decreased heart rate, decreased cardiac output, decreased blood volume
- Increased body weight, constipation, mental sluggishness, depressed growth of hair and dry skin
- Development of a froglike hoarseness of voice and in severe cases, edematous appearance of the body called myxedema.

CARDIOVASCULAR FEATURES OF HYPOTHYROIDISM

The decreased thyroid hormone causes are decreased heart rate, mild increase in BP, decreased pulse pressure, and mildly increased mean arterial pressure, with some amount of exercise impairment **(McAllister et al, 1995: Klein and Ojamaa, 2000)[32].**

Increased QT interval with inverted or flattened T waves are the ECG findings associated with hypothyroidism **(Freudlund and Olsson, 1983: Klein and Ojamaa, 2000)[33]** which shows the prolonged cardiac action potential. Due to increased myocardial electrical dispersion in an already damaged heart disease these individuals are vulnerable candidates for ventricular arrhythmias **(Fredlund and Olsson, 1983: Klein and Ojamaa, 2000)[33].**

Systemic hypertension is also seen more commonly in patients with hypothyroidism **(Endo et al[34], Saito et al[35], Streeten et al,[36] Klein 1989[37] Fletcher and Weetman, [38] Fommei and lervasi et al [39]).** The causes for increased systemic BP are increased arterial wall stiffness and increased peripheral vascular resistance **(Dernellis and Panaretou, 2002: Obuobie et al 2002)[40].**

TREATMENT OF HYPOTHYROIDISM:

Hypothyroidism, either primary or central, is gratifying to treat because of the ease and completeness with which it responds to thyroid hormone. Treatment is nearly always with levothyroxine and the proper use of this medication has been reviewed extensively (**Roti E, Minelli R, Gardini E, et al, Mandel SJ et al, Brent GA et al, Larsen PR et al, Toft et al)[41].**

Hypothyroidism is treated with ease and completeness by replacement of thyroid hormone with Levothyroxine **(Mandel ST and Brent GA et al 1993)[42]**. This type of replacement has got an advantage that the peripheral deiodination mechanisms will produce the necessary amount of T_3 in tissues under the normal physiologic control. The principle of hormone replacement is to replicate the natural state by providing a

prohormone T_4 and allowing the peripheral tissues to activate it by physiologically regulated mechanisms.

Levothyroxine has a 7-day half-life. The dose of levothyroxine is approximately 1.6 to 1.8 μ g /kg ideal body weight per day. Because of this long half –life, missed dose can be taken on the next day. In hypothyroidism, this will usually result in normal range of serum TSH concentration in 6 weeks. Accordingly, assessments of the adequacy of a given dose should be made after 6weeks only.

To summarize, hypothyroidism may be conceptualized, not just as an endocrine disorder affecting all types of tissues of the body but also treatable with adequate replacement with L-thyroxine.

AUTONOMIC NERVOUS SYSTEM

Langley in 1898 coined the term autonomic nervous system (ANS) as a part of the nervous system responsible for homeostasis which innervates all organs except for skeletal muscle which is supplied by somatic motor system.

The two major divisions of ANS are sympathetic (thoracolumbar division) and parasympathetic (craniosacral division). The two divisions work in a coordinated manner to maintain the homeostasis. The enteric nervous system in the GIT also forms a part of ANS. The **preganglionic**

and **post ganglionic neurons** forms the peripheral motor part this nervous system. The axons are small, myelinated, slow conducting B fibers in case of preganglionic neurons with more diverging fibers to eight or nine postganglionic neurons. This forms a diffuse autonomic output. In case of the postganglionic neurons they are mostly unmyelinated C fibers and terminate on the visceral effectors **(Kandel ER and Schwartz JH, et al)[43].** The ratio of preganglionic to postganglionic neurons is smaller (1:15 to 1:20) in contrast to the sympathetic system.

This smaller ratio between pre and post ganglionic neurons helps in more localized and specified nature of this part of ANS. In contrast, the increased ratio of preganglionic and postganglionic neurons correlates with wide spread sympathetic autonomic effects and massive sympathetic outflow during strenuous physical activity and stressful environments.

NEUROTRANSMITTERS IN ANS

The principal neuro transmitter agents involved are **acetylcholine in the parasympathetic nerve endings** and **norepinephrine in the sympathetic nerve endings**. The evidence for this chemical neurotransmission was provided by Otto Loewi in 1920 **(Ganong et al)[12].**

AUTONOMIC INNERVATION OF HEART

The Heart and the peripheral vascular system are innervated by both divisions of ANS. This is controlled by cardiac autonomic centers situated in the medulla, which form the integrating centers of cardiac autonomic reflex. The sympathetic innervation is controlled by cardiac excitatory area in Rostral Ventral Lateral Medulla (RVLM) also known as vasomotor center. The cervical sympathetic nerves form the efferent limb of cardiac autonomic reflex. The parasympathetic center is located in dorsal motor nucleus of vagus and nucleus ambigus and its efferent is the vagus nerve. The afferent inputs from various peripheral and central organs reach these cardiac autonomic centers. Some inputs excite the cardiac sympathetic centers, which in turn inhibits the parasympathetic center. There is an antagonistic effect of these both vital centers on each other.

Distribution of autonomic receptors in heart

1. Acetylcholine Receptors: The acetylcholine receptors have been divided into two types on the basis of their pharmacologic properties. They are Muscarinic and Nicotinic cholinergic receptors. Muscarinic type of cholinergic receptors is found in heart that is mainly M2 subtype. It is a serpentine type of receptor coupled via heterotrimeric G proteins to adenyl cyclase. Here the second messenger involved is the cyclic AMP. This receptor activation results in decreased production of cyclic AMP which

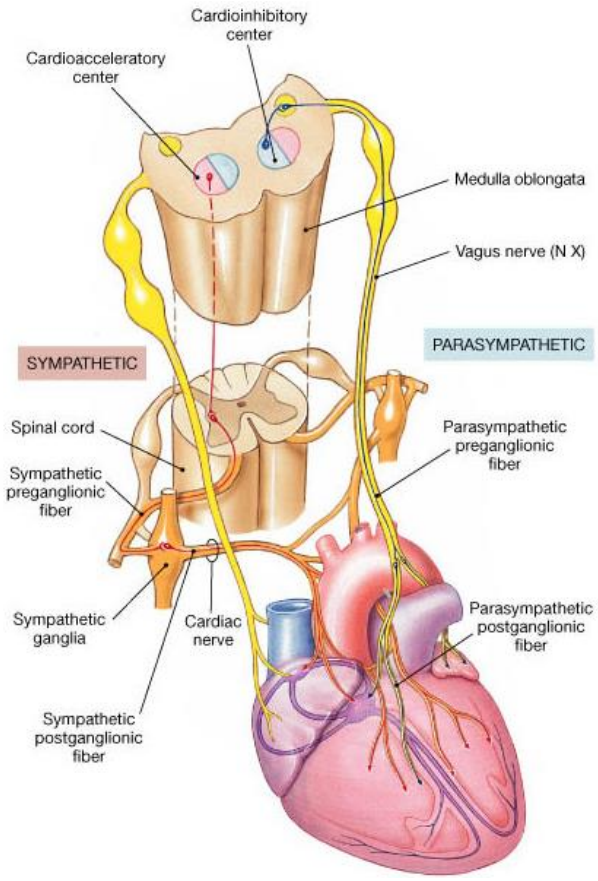


Figure4. Autonomic innervation of Heart

keeps the potassium channels in the open state and allows K+ efflux to occur.

2. Adrenergic receptors: The adrenergic receptors are divided into two types. Most of the postganglionic sympathetic neurons release norepinephrine as neurotransmitter which will act through adrenergic receptors. Exception to this is sympathetic innervation is sweat glands where the postganglionic neurons release acetylcholine and act through muscarinic receptors.

The adrenergic receptors are G protein coupled receptors and highly homologous to the muscarinic receptors. Two major types of adrenergic receptors are Alpha and Beta, each of which exists in multiple subtypes (e.g., α1, α2, β1, β2 and β3). These receptors activate the intracellular signal transduction pathways and acts on membrane channels by increased production cyclic AMP as second messenger. The β1 receptors in the heart activate the Gs heterotrimeric G protein and stimulate adenylyl cyclase, which antagonizes the effects of muscarinic receptors.

Adrenergic receptor subtypes have a tissue-specific distribution. α1 receptors predominate on blood vessels, α2 on presynaptic terminals, β1 in the heart, β2 in high concentration in the bronchial muscle of the lungs and β3 in fat cells. This distribution has permitted the development of many clinically useful agents that are selective for different subtypes and tissues.

MECHANISM OF VAGAL STIMULATION ON HEART:

The neurotransmitter acetylcholine after release from vagal stimulation acts on muscarinic receptors. The permeability of the fiber membranes to potassium is increased by opening the special K+ channels. This will increase the negativity causing hyperpolarization and makes this tissue less excitable. The opening of the Ca+ channels is also slowed.

This hyperpolarization will decrease the resting membrane potential of the sinus nodal fibers to a more negative level of -65 to -75 mv than the

normal level of -55 to -60 mv. So it takes longer time to reach the threshold potential for excitation. Therefore vagal stimulation decreases the rate of rhythmicity of the SA nodal fibers.

There may be an absolute reduction in the sinus rate when the vagus is stimulated tonically in the presence of sympathetic stimulation. This is known as accentuated antagonism. The cardiac response to sympathetic stimulation starts immediately and disperses slowly. But the cardiac response to vagal stimulation begins after a little latter and dissipates quickly. This type of response to vagal stimulation produces rapid dynamic beat to beat variation in heart rate and AV conduction in contrast to slow temporary response of sympathetic stimulation.

When a systolic pressure wave arrives at the baroreceptor sites in the carotid and aortic sinus regions the periodic vagal bursting occurs. This produces changes in duration of cardiac cycle length in a phasic manner allowing the sinus node to discharge at a variable rate that is identical to those of vagal burst. In the same manner vagal bursts also prolong AV nodal conduction time that is influenced by sympathetic tone.

A brief vagal burst can decrease the SA node rate without altering the AV conduction time. Or it may prolong the AV node conduction time but not influencing the sinus node rate. Because in a cardiac cycle the peak vagal effects can occur at different times. This shows that vagus can

have both direct and indirect effect by altering the sympathetic influences **(Levy MN et al, 1994)[11].**

MECHANISM OF SYMPATHETIC STIMULATION ON HEART:

Stimulation of sympathetic nerve releases the neurotransmitter adrenaline at its nerve endings which acts on ß1 receptors causing rapid opening of Ca+ channels. Accelerating the rate of depolarization and slope of the prepotential increases the heart rate. The rate of impulse formation in the SA node is increased by stimulation of right stellate ganglion. The rate of conduction in the AV node is increased by left stellate ganglion.

Acetylcholine increases the refractory period and Norepinephrine decreases the refractory period in the center of sinus node. Acetylcholine binds with the M2 receptors in the sinus node. After binding a specific G_1 protein is stimulated. This in turn activates the K channel and will modulate the discharge rate. Inhibition of adenylate cyclase via G_1 also antagonizes the adrenergic effects on the sinus node. After the end of vagal stimulation, sinus node discharge rate may automatically accelerate transiently which causes post vagal tachycardia **(Bouman LN et al, 1978)[44].**

CENTRAL CONTROL OF AUTONOMIC FUNCTION:

Higher centers comprising of hypothalamus, brainstem, limbic system have vital connections with the preganglionic neurons to integrate and regulate internal visceral functions to maintain homeostasis.

Because of the major role of the hypothalamus in autonomic function, it has at times been labeled as the “Head ganglion of the ANS.” The sensory and motor areas involved in control of autonomic function includes the insular and medial prefrontal areas of the cerebral cortex. The autonomic components of emotional responses are regulated by amygdala in the temporal cortex. The areas of the cerebral hemispheres, diencephalon, brainstem and central pathways to the spinal cord that are involved in the control of autonomic functions are collectively termed the central autonomic network.

AUTONOMIC DYSFUNCTION IN HYPOTHYROIDISM

There are several studies reflecting the role of ANS in hypothyroidism some showing sympathetic activity and others showing parasympathetic activity.

Inukai et al [45] on evaluated the parasympathetic nervous activity in hypothyroid individuals by determining the RR interval variations on Electrocardiogram. He observed a reduction in RR interval variations in patients with primary hypothyroidism. There are also decreased parasympathetic activities with reduced levels of thyroid hormones.

Also he observed the Power spectral analysis of hypothyroid patients with Hashimoto’s thyroiditis showed decreased LF/HF ratio than the healthy subjects.

Kahaly et al (2000)[46] observed in his study on cardiovascular system and atherogenic aspects of subclinical hypothyroidism that decreased Heart Rate Variability can be appreciated in subclinical hypothyroidism even with little changes in the levels of thyroid hormones. He also reported that RR interval variations were restored to normal after treatment.

Matsukawa et al (1993)[47]**,** observed the change in muscle sympathetic nerve activity in thyroid disorders. He observed that there may be a positive correlation of TSH and muscle sympathetic nerve activity. This denotes an inverse relationship between thyroid gland function and sympathetic nerve activity.

Momose et al (1997)[48] demonstrated that increased cardiac as well as generalized sympathetic activity in hypothyroid individuals by using Iodine-123 metaiodobenzylguanidine scintigraphy.

Guasti et al[49] suggested that there may be differences in local concentrations of NE or altered post-receptor signaling in patients with thyroid dysfunction. He observed that after treatment there was a reduced sympathetic activity in hypothyroid patients.

Fommei et al (2002)[50] in his study on role of thyroid hormone on blood pressure homeostasis found that decreased thyroid hormones may

cause an increase in BP as well as excitation of sympathetic system. This increased BP was seen to be reducible with thyroid hormone replacement.

Galetta et al (2008)[51] on evaluating the subclinical hypothyroid patients with heart rate variability and QT dispersion observed a decrease in sympathovagal modulation of heart rate with an increased QT intervals.

Cacciatori V, Gemma ML, et al (2000)[52] observed lowered total power in hypothyroid patients on HRV analysis of heart rate in hypothyroidism.

Sahin I, Turan N, Kosar F et al (2005)[53] evaluated autonomic balancey in subclinical hypothyroid patients and observed significantly lowered total power in untreated subclinical hypothyroid individuals.

Xing H et al (2001)[54] showed significantly lower values of HF power and HF norm in untreated hypothyroidism indicating their reduced vagal modulation of heart compared to euthyroid and treated hypothyroid patients who obtained euthyroid status.

Bhat AN et al[55] found increased sympathetic activity in hypothyroid compared to normal controls in their study.

Coulombe P et al (1976)[56] by studying the secretion rate of epinephrine in thyroid disorders found that increased sympathetic activity and decreased vagal modulation can occur in hypothyroidism. But

bradycardia seen in hypothyroidism may be due to receptor desensitization. Also there may be decreased chronotropic response to inspite of increased sympathetic activity.

Polikar R et al, Kennedy B et al, Maisel A et al, Ziegler M et al (1990)[57] in their study also observed the similar findings as above in hypothyroidism.

Ahmed M, Begum N, Ferdousi S, et al, (2010)[58] found alteration in cardiac autonomic activity characterized by decreased vagal activity and increased sympathetic activity in hypothyroidism by doing power spectral analysis.

Manhem P, Bramnert M, Hallengren B et al (1992)[59] showed raised plasma levels of catechol amines in hypothyroidism with decreased adrenergic responses at cardiac and peripheral level. This may indicate pre and post receptor desensitization.

Heemstra KA et al, Burggraaf J et al, Vander Klaauw AA et al[60] observed metabolic actions of thyroid hormone. There may be an increased protein deposition in extracellular space causing increased water content in myocardial wall. There is fibrosis which will lead to increased inhomogeneity of ventricular repolarization. Moreover they also observed opposing effects on circulating lipids, swelling of vascular smooth muscle

and altered endothelial cell function in their study involving the effect of short term overt hypothyroidism on sympathovagal balance in thyroidectomy done patients of differentiated thyroid carcinoma.

Akcakoyun M, Emiroglu Y, Pala S, et al (2010)[61] also reported a hypo functional parasympathetic system based on analysis of heart rate recovery and RR variations in ECG in patients of subclinical hypothyroidism.

Luboshitzky R, Aviv A, Herer P, Lavie Let al (2002)[62] on analyzing the various complicating factors for heart disease in women with subclinical hypothyroidism observed raised diastolic blood pressure in these individuals.

The current study proceeds with studying the autonomic system functioning in hypothyroid females before and after therapy with L-thyroxine.

HEART RATE VARIABILITY:

Heart rate variability refers to the beat - to - beat variation in heartbeat. Even in a healthy individual, the resting heart beat is not strictly regular, an observation described by **Albrecht Von Haller** in the 18th century. The duration of cardiac cycle varies from beat to beat and this spontaneous variation of RR interval in milliseconds from beat to beat is

known as Heart rate variability. Intrinsic automaticity of the sinoatrial node and modulating influence of the autonomic nervous system determines the rate and rhythm of heart. Under resting conditions, the vagal or parasympathetic tone predominates.

. The variations in heart rate are largely dependent on vagal modulation, which releases acetylcholine on stimulation. There will be release of epinephrine or norepinephrine on stimulation of sympathetic nerves in the heart **(levy MN et al, 1971)[63].** Heart rate variability is the conventional term used to describe variation of both instantaneous heart rate and R-R intervals. The other terms used to describe are cycle length variability, heart period variability, R-R interval tachogram, R-R variability. This more precisely explains that this interval is between two successive heart beats rather than the heart rate.

The physiological basis for this heart rate variability originates from fluctuations in the cardiovascular vasoconstrictor and vasodilator centers in the brain. These fluctuations usually arise due to baroreflex mediated changes in blood pressure, respiration, thermoregulation and circadian rhythm. All these oscillators will have an influence over the length of RR interval.

The balance between the sympathetic and parasympathetic system is constantly changing to achieve optimum in response to various internal and external stimuli and to maintain the homeostasis. The increased HRV

indicates the more flexible and quicker response of the heart to various internal and external influences. Decreased HRV reflects the dysfunction of autonomic nervous system as well as low HRV may indicate a reduced capacity for adaptation and may suggest health impairment **(Sampson MB, Mudaliar NA, Lele AS; 1980)[64].**

With normal accepted range of other physiological parameters, HRV can reflect changes in body stress. Some HRV changes might be a first sign of distress, reflecting involvement of energy more dependent sympathetic system. The decrease in biological signals variability is a warning sign of loss of self-regulating homeo kinetic mechanisms.

Heart rate variability (HRV) is a measure of the respiratory sinus arrhythmia in which the heart rate accelerates during inspiration, and decelerates during expiration, in which the R-R interval in an ECG is shortened during inspiration and prolonged during expiration and is due to oscillations in sympathetic nerve discharge synchronized with respiratory rhythm. This is a normal phenomenon, which may decrease with age and also under stressful conditions.

Animal studies have shown that HRV is considered as a marker of vagal activity **(Brouha and Nowak, 1939)[65].** This can be evidenced in humans by blocking the parasympathetic system by atropine **(Bernston et al, 1994)[66]** which indicates that HRV is a function of parasympathetic activity.

The clinical relevance of HRV was first appreciated by noting the fact that fetal distress was preceded by alterations in inter beat intervals before any appreciable change occurred in heart rate itself **(Hon and Lee et al; 1965)[67].** Attention was focused on the existence of physiological rhythms imbedded in the beat-to-beat heart rate signal by Sayers and others to detect the autonomic dysfunction in diabetic patients**. Ewing et al[68]** described some simple bedside tests of short-term HRV. The increased risk of relation between post infarction mortality and reduced HRV was first observed by **(Wolf et al; 1977)[69].** Power spectral analysis of heart rate fluctuations was introduced by **(Akselrod et al; 1981)[70].**

Stein P.K. et al (1994)[71] recommended that the activity of sympathetic and parasympathetic innervation on sinus node of heart can be measured with heart rate variability as it is a non-invasive cardio graphic marker.

Among the various tests, the resting Heart rate variability can be used as the noninvasive test to evaluate the sympathovagal balance at sinoatrial node and functional state of ANS **(Juan Sztajzel et al, 2004)[72].**

In last 30 years various studies were conducted which showed the relationship between autonomic nervous system activity (ANS) and sudden death in cardiac arrhythmias **(Levy et al, 1994)[73].**

COMPONENTS OF HEART RATE VARIABILITY:

During resting state vagal tone is predominant and variation in heart period is mostly dependent on vagal modulation. Vagal and sympathetic activity constantly interacts with each other. The R-R interval variation present during rest represents a control mechanism which finely tunes the heart rate from beat to beat. Afferent vagal stimulation results in efferent vagal activity and inhibition of sympathetic activity.

The opposite reflex occurs by stimulation of afferent sympathetic activity. The efferent vagal activity appears to be under "tonic" restraint by cardiac sympathetic activity. Efferent sympathetic and vagal activity directed to the sinus node and characteristic discharge from it, is largely synchronizing with each cardiac cycle. And it can be modulated by central (vasomotor and respiratory center) and peripheral (arterial pressure and respiratory movements) oscillators.

These oscillators generate rhythmic fluctuations in efferent neural discharge that manifest as short and long term oscillations in heart period. **(Task Force 96)[74].** Analysis of the rhythm would reveal as about the

1) Central oscillatos

2) Sympathetic and efferent vagal activity

3) Humoral factor

4) Sinus node activity.

CLINICAL USES OF HRV:

- HRV is the non-invasive simple test for measuring both cardiovascular and non-cardiovascular autonomic function.
- To evaluate the treatment effectiveness and prognosis
- HRV is used for exercise training in sports physiology
- To assess the effect of various stress relaxation programs like massage, exercise, meditation, yoga
- HRV analysis is a predictor of risk after MI, arrhythmias
- Can be used to find out autonomic dysfunction in diabetes mellitus patients.

Conny MA et al[75] suggested the two types of heart rate variability assessment:

1. Time domain methods – the various indices were calculated by statistical methods on RR intervals.

2. Frequency domain methods - by spectral analysis of RR intervals. The accurate timing of R waves is must in both these methods. The analysis can be done both on short term ECG segments which will last for 0.5-5 minutes or on 24 hour ECG recordings. Rapid changes in heart rate is indicated by short-term variability (STV) indices.

In HRV analysis either the heart rate as a function of time or the intervals between successive QRS complexes need to be determined. In this review the term HRV actually means the variability of RR intervals (i.e. intervals between consecutive R peaks). We get RR intervals through ECG in milliseconds. By using the specific software algorithms the recorded ECG signal can be processed in such way that the regular heart beat pattern can be determined.

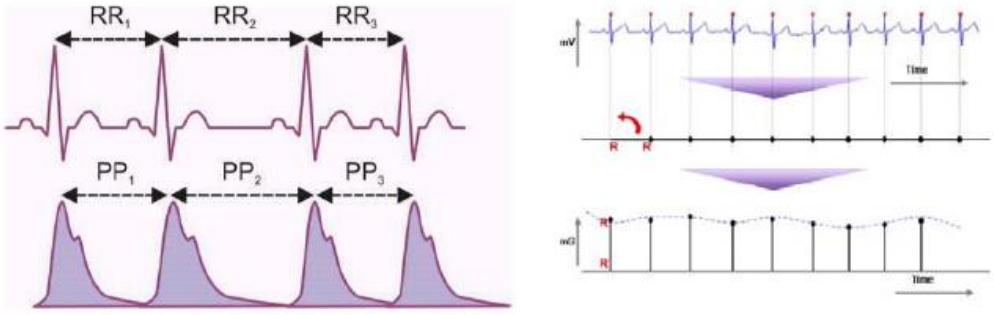


Figure 5: Variability of RR Interval.

R-R interval: The duration of time between two subsequent R wave peaks is called as the R-R interval. The duration of the RR interval is determined by the combined effect of sympathetic and parasympathetic tones. If a particular RR interval becomes longer than preceding one, it indicates a relative increase in vagal tone compared to the sympathetic tone during that period. Similarly increase in sympathetic tone or decrease in parasympathetic tone is reflected as reduced RR interval or an increased heart rate **(Philip A. Low 1997).** It is usually expressed in seconds or

milliseconds. Among the limb leads, the R-R interval obtained in ECG recording of lead II is specific.

The two methods used to measure heart rate variability on the basis of R-R interval fluctuations are

- Time domain methods
- Frequency domain methods

TIME DOMAIN METHODS:

The time domain methods are simplest to perform since they are applied straight to the series of successive RR interval values.

By a continuous ECG record, the heart rate at any given point in time or the interval between two successive normal complexes is determined. Different variables measured includes

Variable	Units	Description
Statistical Measures		
SDNN	ms	Standard deviation of all NN intervals
SDANN	ms	Standard deviation with respect to the averages NN intervals in all 5-minute segments for the entire recording
RMSSD	ms	Square root of the mean of the sum of the

		squares of differences between adjacent NN intervals
SDNN index	ms	Mean of the SD of all NN intervals of all 5-minute segments for the entire recording
SDSD	ms	Standard deviation of differences between adjacent NN intervals
NN50 count		Number of pairs of adjacent NN intervals differing by more than 50 ms in the entire recording; three variants are possible counting all such NN intervals pairs or only pairs in which the first or the second interval is longer
pNN50	%	NN50 count divided by the total number of all NN intervals

Every QRS complex is recorded and NN interval (complete intervals among contiguous QRS complex is determined.

Other commonly used variables are Mean HR, Mean RR.

FREQUENCY DOMAIN METHODS

In this method, for the RR intervals, a power spectrum density (PSD) estimate is calculated. Then the RR interval series is changed to uniformly distributed or distanced sampled series by interpolation methods prior to

PSD estimation. FFT based methods or parametric AR modeling based methods are usually used for PSD estimation. FFT based methods have the merits due to the simplicity of implementation, though the AR spectrum yields improved resolution particularly on lesser samples size.

Variable	Units	Description	Frequency Range
Analysis of Short-term Recordings (5 min)			
5-min total power	ms^2	The variance of NN intervals over the temporal segment	≈≤0.4 Hz
VLF	ms^2	Power in VLF range	≤0.04 Hz
LF	ms^2	Power in LF range	0.04-0.15 Hz
LF norm	Nu	LF power in normalized units LF/(total power−VLF)×100	
HF	ms^2	Power in HF range	0.15-0.4 Hz
HF norm	Nu	HF power in normalized units HF/(total power−VLF)×100	
LF/HF		Ratio LF [ms^2]/HF[ms^2]	

VLF (0-0.04Hz):

Primarily indicates sympathetic activity, renin angiotensin system activity, baroreflex activity and thermo regulation.

Not relevant in 5min ECG recordings**.**

LF power (0.04-0.15Hz)

LF shows baroreflex mediated blood pressure maintenance of the sympathetic system.

This is an indicator for more sympathetic than parasympathetic modulation. This needs minimum of 2 minutes ECG recording**.**

HF power (0.15-0.4Hz):

HF power reflects parasympathetic activity mainly like gas exchange efficiency and RSA, which are mediated by vagus nerve.

Even 1 minute ECG recording is enough

ULF- less than 0.003 Hz

Mainly used for analyzing long term recordings (24 h), becomes relevant clinical significance is not clearly defined.

The standard deviation of the mean RR interval is a commonly determined cardiovascular autonomic function. The standard deviation provides a statistical measure of the variability or spread of the RR intervals around the average heart rate. The results of this determination depend on

the number of observations (RR intervals) and the mean Heart rate **(Smith SE and Smith SA et al, 1981)**[76].

It is proposed that a measure based on successive differences in the RR intervals, that are the actual difference between adjacent RR intervals would be more sensitive than the standard deviation to short term fluctuations in heart rate. The mean square successive difference (MSSD) is the average of the square of the differences between successive beats; the RMSSD is its square root **(Gundersen and Neubauer et al, 1981)**[77]

In this study we are using both Time domain and Frequency domain methods of resting heart rate variability analysis to determine the sympathovagal balance in hypothyroid female subjects and the changes observed with L-thyroxine therapy.

ECHOCARDIOGRAPHY IN HYPOTHYROIDISM

Cardiovascular imaging with Echocardiography plays an important role in evaluating structural and functional status of the heart. The Two-dimensional (2D) echocardiography helps to view the heart directly with ultra-sonogram. This gives on the spot assessment of the myocardium, chambers, heart valves, vessels and pericardium. Doppler study gives the velocity of moving red blood cells. This can be used to assess of hemodynamics non invasively replacing cardiac catheterisation.

The principle behind 2D echocardiography is reflection of ultrasonic waves from cardiac structures that produces images of the heart for a transthoracic echocardiogram (TTE). Recent ECHO machines are portable. So the key merits of echocardiography over other modalities are the capability in obtaining instantaneous images of the heart structures for swift interpretation.

A constraint of TTE is the difficulty in obtaining better-quality images of patients with a thick chest wall or severe lung disease, due to the poorly transmission of ultrasound waves through lung parenchyma. 2D echocardiography is also the perfect imaging modality for analysing left ventricular (LV) size and function. A qualitative assessment of the ventricular cavity and systolic function can be made directly from the 2D image. LV hypertrophy and hypertrophic cardiomyopathy can be diagnosed with 2D echocardiography. Others like left atrium and right champers assessed by visual analysis.

Cardiovascular disorders associated with hypothyroidism include decrease in myocardial contractility, pericardial effusion, and increase in left ventricular mass and prolonged duration of contraction and relaxation. The ejection fraction, cardiac reserve are slightly diminished **(Stiefelhagen P et al 2009)**[78] The various clinical studies on cardiac involvement in subclinical hypothyroidism consistently shows that patients exhibit resting left ventricular diastolic function evidenced by delayed relaxation, and

impaired systolic function on effort that results in poor exercise capacity (**Rodondi N et al, (2007), Aujesky D et al, Vittinghoff E et al 2006)**[79]. Most of these cardiac manifestations are reversible with timely and adequate therapy.

The various echocardiographic parameters that are assessed includes left ventricular posterior wall thickness(LVPW), Interventricular septum(IVS), Ejection fraction(EF%)-for LV systolic function, cardiac chamber size mainly LVID –left ventricular internal diameter both end systolic(ES) and end diastolic(ED).

As a non –invasive method, echocardiography can play an important role in recognizing the cardiac pathology as well as to follow up effect in the therapy.

There is alteration of myocardial wall thickness in hypothyroidism as shown by various studies. **Bennet et al (1983), Lee et al (1990)**[80]**, Bernstein et al (1995)**[81] reported considerable increase in incidence of asymmetrical septal or concentric hypertrophy in these patients. The cause increased wall thickness in hypothyroidism could be due to altered peripheral vascular resistance in these patients.

There are also decreased systolic functions in hypothyroidism as observed by **Forfar et al (1992)**[82]. The cause for reduced cardiac output may be decreased stroke volume and heartrate. The decreased cardiac index

in hypothyroidism could be reversed with thyroid hormone replacement as per **Kral et al** (1992)[83].

This study uses the Echocardiographic parameters like ejection fraction and LVID as a measure of functional state of the heart in normal and hypothyroid females before and after treatment with L–thyroxine replacement therapy.

AIM & OBJECTIVE

OF THE STUDY

AIM AND OBJECTIVE OF THE STUDY

AIM OF THE STUDY

To assess the Resting Heart Rate Variability and Echocardiography in normal and hypothyroid (female) patients before and after treatment.

OBJECTIVES OF THE STUDY

1. To analyze the Resting Heart Rate Variability in newly diagnosed hypothyroid (female) individuals before treatment and normal healthy females.

2. To assess the cardiac functional status by doing Echocardiography in newly diagnosed hypothyroid (female) individuals before treatment and normal healthy females.

3. To compare the changes in Heart Rate variability and Echocardiography before and after treatment with thyroxine in hypothyroid female individuals.

MATERIALS AND METHODS

MATERIALS AND METHODOLOGY

This study was conducted in the Institute of Physiology and Experimental Medicine, Madras Medical College, Chennai in association with the Medical Endocrinology Outpatient Department, Rajiv Gandhi Government General Hospital, Chennai, after obtaining the ethical committee clearance.

The study was conducted in the period of September 2014 to August 2015.

The study group consists of 30 female subjects who were newly diagnosed hypothyroid individuals in the age group 20-35 years. The control group includes of 30 age matched, euthyroid females for comparison with the cases.

The subjects were informed about the study details, procedures and their acceptance to participate in the study was obtained as written consent from them.

INCLUSION CRITERIA

Newly diagnosed hypothyroid females with estimated (serum TSH levels above 10mIU-100mIU/ml with both normal and decreased serum T_3, T_4 levels) in the age group of 20-35 years are included in this study as cases.

For the purpose of statistical analysis, the study subjects are considered as following groups**:**

Group A : Normal control

Group B_{BT} : Hypothyroid (newly diagnosed cases, before starting treatment)

Group B_{AT} : Hypothyroid (after treatment with L-thyroxine for minimum 3months and who have attained euthyroid status with therapy)

EXCLUSION CRITERIA

Pregnant females, subjects taking oral contraceptives or drugs affecting heart rate, subjects with anemia (Hb less than 10gm/dl), hypertension, diabetes mellitus, underlying known respiratory, cardiac, renal, hepatic, neoplastic conditions, and other concurrent medical illness are excluded from the study**.**

MATERIALS

- Niviqure ambulatory digital ECG recorder (INCO)
- Sphygmomanometer
- Stethoscope
- Philips HD 7 Diagnostic ultra sonographic machine (2D ECHO)

Estimation of serum T_3, T_4, TSH levels:

Serum analysis of T_3, T_4, TSH levels were done in Biochemistry Laboratory at Rajiv Gandhi Government Hospital. The T_3, T_4, TSH levels are measured using ELISA method. As a parameter for statistical analysis the serum TSH levels only taken.

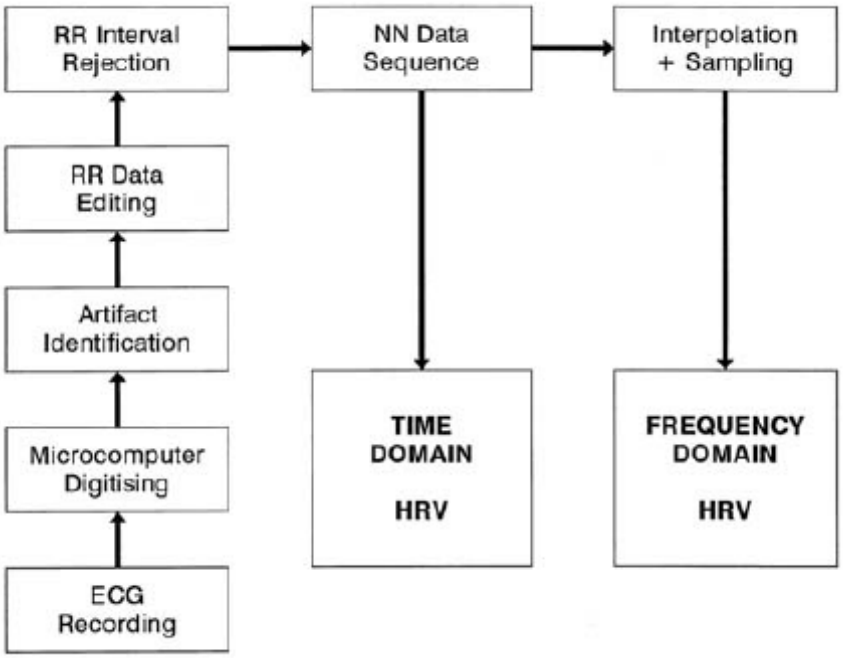
HRV ANALYSIS

Niviqure Ambulatory Digital ECG recorder

Niviqure is a solid state, multi load, digital, standalone computerized recording system designed to acquire, analyze and ECG data over long period. This data is acquired and stored in flash memory for later down loading and analysis. The data transfer from memory module and computer is through an interface Rs233C, compatible module.

Niviqure has powerful processing software for online ECG study, data storage, off line data replay and study and data transfer to other software for statistical analysis and FFT analysis.

Flow chart given below shows the basic steps used to record and process the ECG signal to obtain data for HRV analysis.



METHODOLOGY

The subjects were asked to fill a proforma to assess the general status and clinical symptoms suggestive of hypothyroidism.

After getting an informed and written consent from the subjects, general and systemic examination were carried out and height in meters and weight in kilograms were also measured.

Body mass index (BMI) was calculated using the formula weight/height in m^2. The short term Resting HRV analysis for 5 minutes was recorded in the subjects.

Short term HRV analysis

As per the recommendations of the Task force of the European Society of Cardiology and the North-American Society of Pacing and Electrophysiology in 1996, the short term HRV recording was done for 5 minutes.

Pretest instructions were given to the subjects as below:

- The subjects were advised to have a good sleep at night before the examination day.
- No heavy activity 24 hours prior to recording.
- Advised to avoid tea, coffee like drinks on the day of testing
- Mobile phones must be switched off

- The subjects were asked to empty the bladder before the test
- The subjects should be relaxed, comfortable and free from recent acute illness, and without significant anxiety.

The test should be performed in a quiet room with lighting sub duded, temperature controlled and sound proofed, well electrified room.

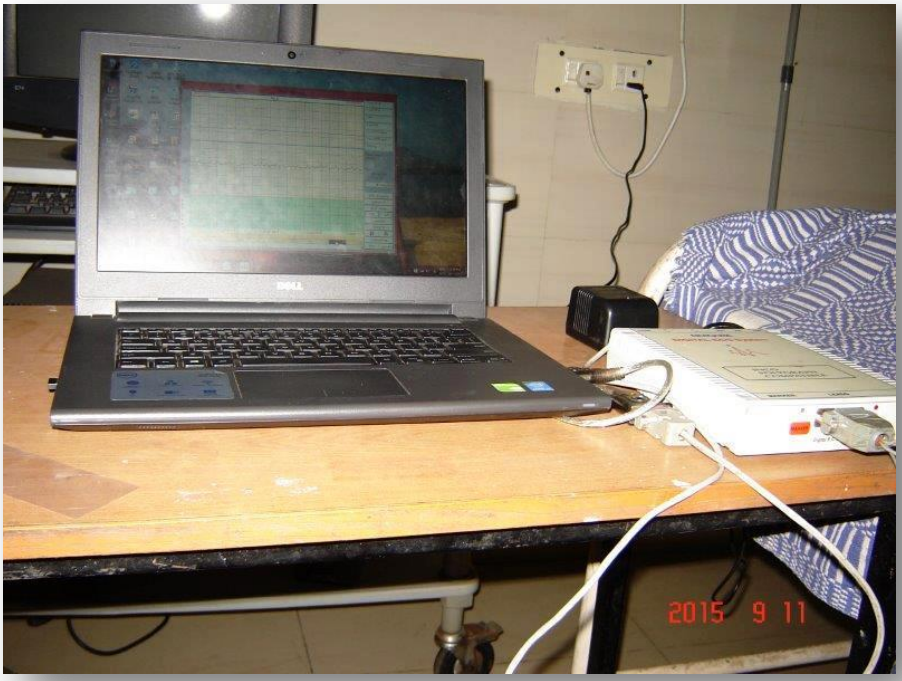
The subject was made to rest quietly in supine position in a cool and calm environment, for a minimum period of twenty minutes.

Electrodes were placed in the following positions after cleaning the site with spirit cotton and connecting the channels to transducer for ECG with computer the HRV was recorded.

EXPLORING ELECTRODE	RIGHT SHOULDER
EXPLORING ELECTRODE	LEFT SHOULDER
EXPLORING ELECTRODE	LEFT SUBCOSTAL
REFERENCE ELECTRODE	RIGHT SUBCOSTAL

The rest period was increased to 30 minutes and then ECG was acquired by continuous ECG recording for five minutes (320 seconds) which is needed for short term HRV analysis. After screening for the artefact and editing it, the results were fed to HRV analysis software (Kubios).

1. Niviqure Digital ECG system



2. Resting HRV Analysis 1

3. Resting HRV Analysis 2

The analogue to the digital conversion of the resting ECG signal was done using AD converter with sampling frequency of 1024/sec. Power spectral analysis of the converted ECG signal was done using Fast Fourier Transformation.

Mean RR, standard RR, Mean HR, standard HR, SDNN, RMSSD, Low Frequency, High Frequency, LF/HF ratio, Total power were estimated.

ECHOCARDIOGRAPHY

Echocardiography measurements were performed using 2 dimensional mode, Philips HD 7 diagnostic ultra sonographic machine in the Department of Cardiology. As a parameter of systolic function we measured the left ventricular ejection fraction. Normal reference range of ejection fraction is ≥ 55%. The left ventricular internal diameter (end diastolic) of 3.9-5.3 cm is taken as the normal reference range in women. **(Harrison's principles of Internal Medicine, 18th Edition).**

Other parameter assessed is the presence of absence of pericardial effusion. After measuring all these parameters the study subjects were advised treatment with L-thyroxine in a minimum dose of 25µg up to 100µg/day according to the serum TSH levels.

At the end of 3 months of treatment serum TSH levels are again measured and after attaining the normal serum TSH levels or euthyroid

state, the short term HRV analysis and Echocardiographic measurements were taken again.

By using the same methodology, the above said estimation of serum TSH, Short term HRV analysis, Echocardiographic measurements were done in the normal controls. Then the study group (newly diagnosed hypothyroid) were started on L-thyroxine therapy for 3 months at a dose of minimum 12.5µg-100µg once daily according to the serum TSH estimation.

After 3 months of therapy serum TSH levels are estimated and after attaining normal euthyroid levels, the short term Resting Heart rate variability analysis and Echocardiography were repeated. The results were analyzed for both the controls and study group before and after therapy. Statistical analyses of the data were done using paired and unpaired t tests.

RESULTS

RESULTS

The statistical analysis of the data obtained from conducting the Heart Rate Variability analysis and Echocardiographic study in normal and hypothyroid female individuals before therapy and after attaining euthyroid status with L thyroxine were done using the Statistical Package for the Social Sciences (SPSS) software version 21. The Mean and Standard deviation of the variables were determined for the three groups namely Group A, Group B_{BT} , Group $A_{AT.}$ The unpaired't test was done between normal and hypothyroid female individuals before starting therapy with L thyroxine. And 'paired't test was done among hypothyroid female individuals before and after therapy with L thyroxine. Both tests were employed as the Test of significance at 95% confidence interval. P value <0.05 was considered as significant.

In the following section, the 3 groups of study subjects will be referred to as follows:

Group A : Normal control

Group B_{BT} : Hypothyroid (newly diagnosed cases, before starting treatment)

Group B_{AT} : Hypothyroid (after treatment with L-thyroxine for minimum 3months and who have attained euthyroid status with therapy)

TABLE-I

Comparison of BMI among normal and hypothyroid individuals (before therapy)

Study groups	N	Mean ± SD	P value
Group A	30	23.62 ± 1.12	0.000***
Group B_{BT}	30	25.97 ± 1.37	

P value *- significant **- highly significant ***- very highly significant

TABLE- I compares the BMI among normal and hypothyroid individuals before therapy with L-thyroxine. This was very highly significant.

TABLE-II

Comparison of resting parameters among study groups

Parameters	Study groups	N	Mean ± SD	P value
Resting HR/min	Group A	30	77.73 ± 2.89	0.000***
	Group B_{BT}	30	72.30 ± 5.97	
	Group B_{BT}	30	72.30 ± 5.97	0.004*
	Group B_{AT}	30	77.66 ± 8.12	
Resting SBP/mmHg	Group A	30	117.00 ± 5.68	0.085
	Group B_{BT}	30	119.80 ± 6.69	
	Group B_{BT}	30	119.80 ± 6.69	0.613
	Group B_{AT}	30	119.00 ± 7.34	
Resting DBP/mmHg	Group A	30	74.80 ± 3.39	0.003*
	Group B_{BT}	30	77.87 ± 4.23	
	Group B_{BT}	30	77.87 ± 4.23	0.006*
	Group B_{AT}	30	75.00 ± 3.43	

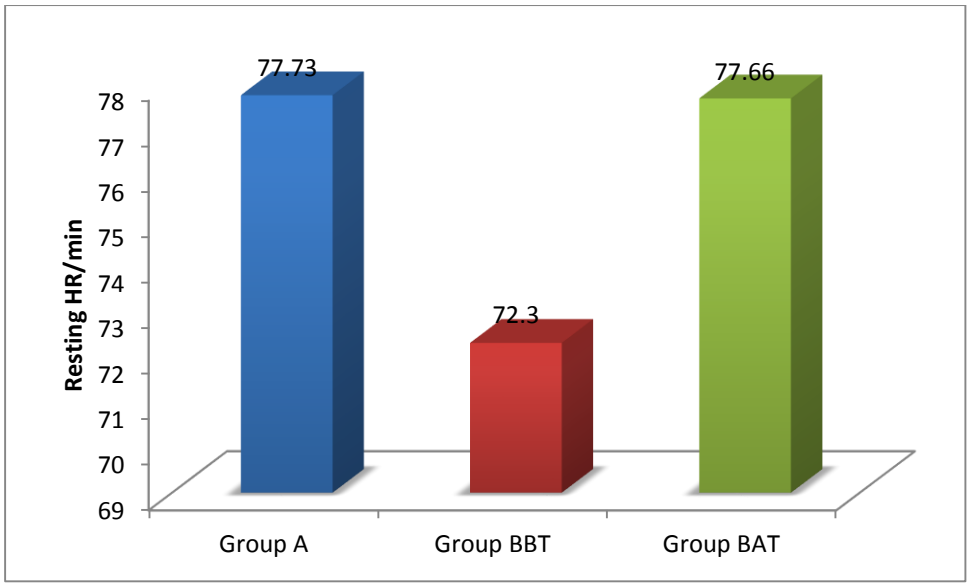
* - significant **- highly significant ***- very highly significant

TABLE- II compares the Resting HR/min, Resting SBP and Resting DBP among the study groups. The mean value for Resting Heart rate in normal and hypothyroid individuals before therapy was highly significant. The mean value of Resting HR in hypothyroid individuals before and after therapy was significant.

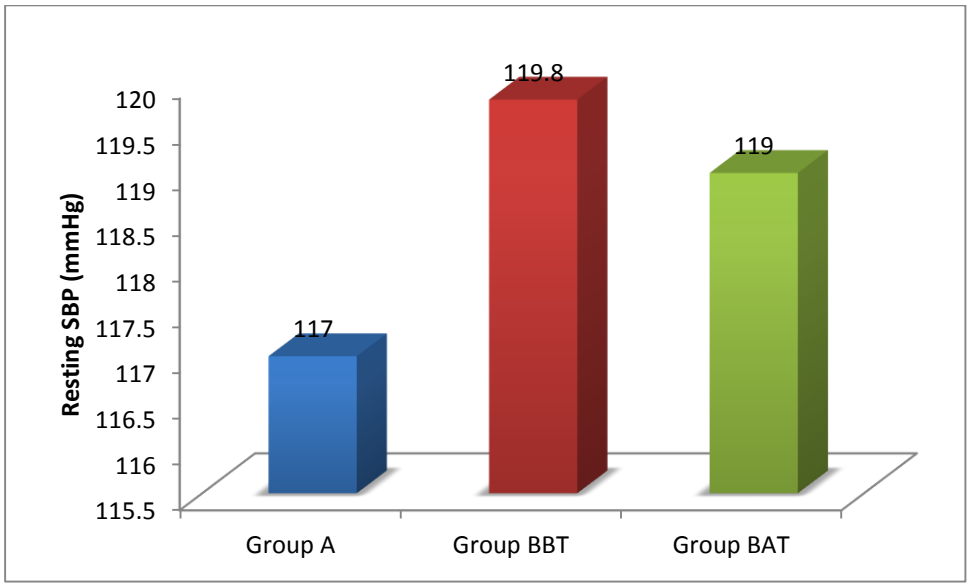
The mean value or Resting SBP among study groups was not significant. The mean value for Resting DBP in normal and hypothyroid individuals before therapy and after L- thyroxine therapy was significant.

Graph 1: Comparison of resting parameters among study groups

1a.Resting Heart Rate/min



1b.Resting SBP (mmHg)



1c. Resting DBP (mmHg)

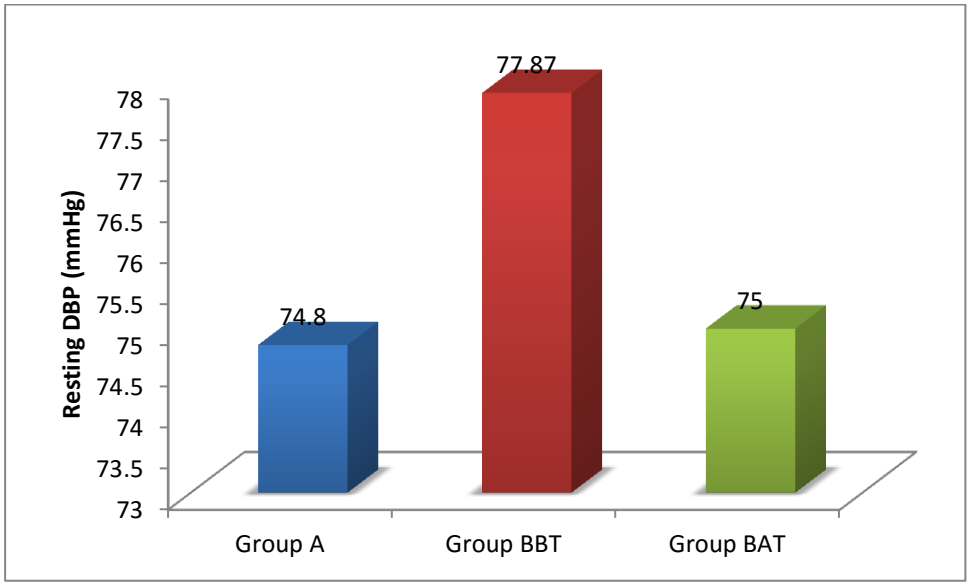


TABLE-III

Comparison of Serum TSH in mIU/ml, among study groups

Study groups	N	Mean ± SD	P value
Group A	30	3.30 ± 1.01	0.000***
Group B_{BT}	30	29.42 ± 12.50	
Group B_{BT}	30	29.42 ± 12.50	0.000***
Group B_{AT}	30	4.22 ± 0.96	

***- very highly significant

TABLE-III shows the comparison of serum TSH levels among study groups. The mean value of serum TSH levels between normal and hypothyroid individuals before treatment was very highly significant. And the mean value of serum TSH levels among hypothyroid individuals before and after L-thyroxine therapy was also very highly significant.

Graph.2. Comparison of Serum TSH (mIU/ml) levels among study groups

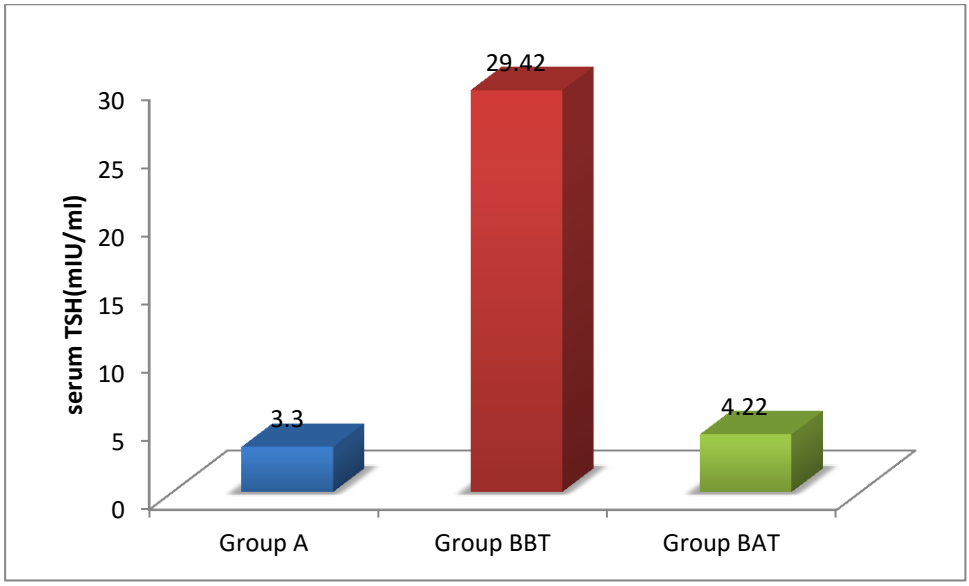


TABLE-IV **Comparison of ECHO parameters among study groups**

Parameters	Study groups	N	Mean ± SD	P value
Ejection Fraction in %age	Group A	30	59.70 ± 2.96	0.000***
	Group B_{BT}	30	49.67 ± 2.95	
	Group B_{BT}	30	49.67 ± 2.95	0.000***
	Group B_{AT}	30	58.36 ± 3.69	
LVID (End Systolic) in cms	Group A	30	2.78 ± 0.19	0.000***
	Group B_{BT}	30	3.81 ± 0.23	
	Group B_{BT}	30	3.81 ± 0.23	0.000***
	Group B_{AT}	30	2.67 ± 0.23	
LVID (End Diastolic) in cms	Group A	30	4.56 ± 0.26	0.000***
	Group B_{BT}	30	4.97 ± 0.31	
	Group B_{BT}	30	4.97 ± 0.31	0.000***
	Group B_{AT}	30	4.52 ± 0.25	

***- very highly significant

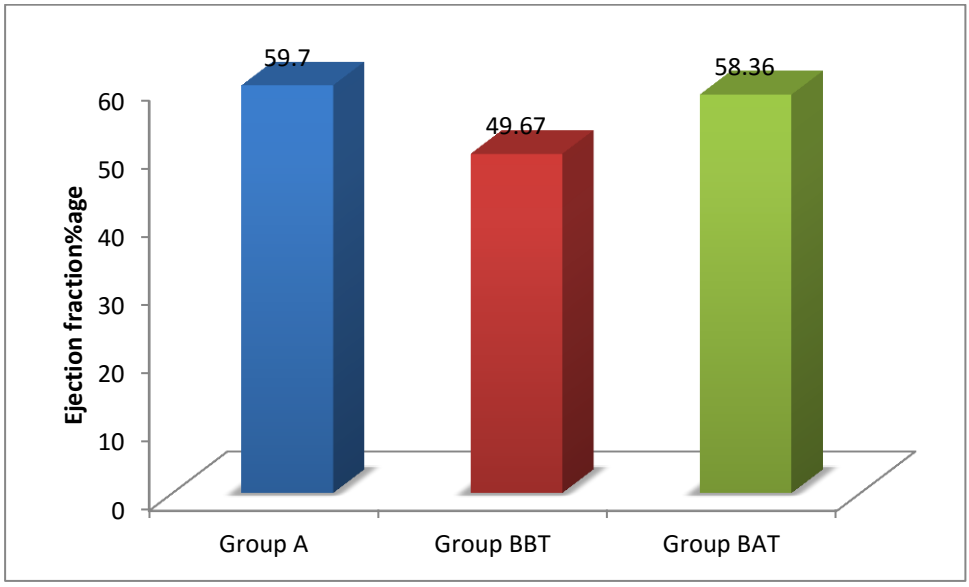
TABLE-IV shows the comparison of ECHO parameters among study groups. The mean value of Ejection fraction between normal and hypothyroid individuals before treatment was very highly significant. And the mean value of Ejection fraction among hypothyroid individuals before and after L-thyroxine therapy was also very highly significant.

The mean value of Left Ventricular Internal Diameter (End systolic) between normal and hypothyroid individuals before treatment as well as after therapy was very highly significant.

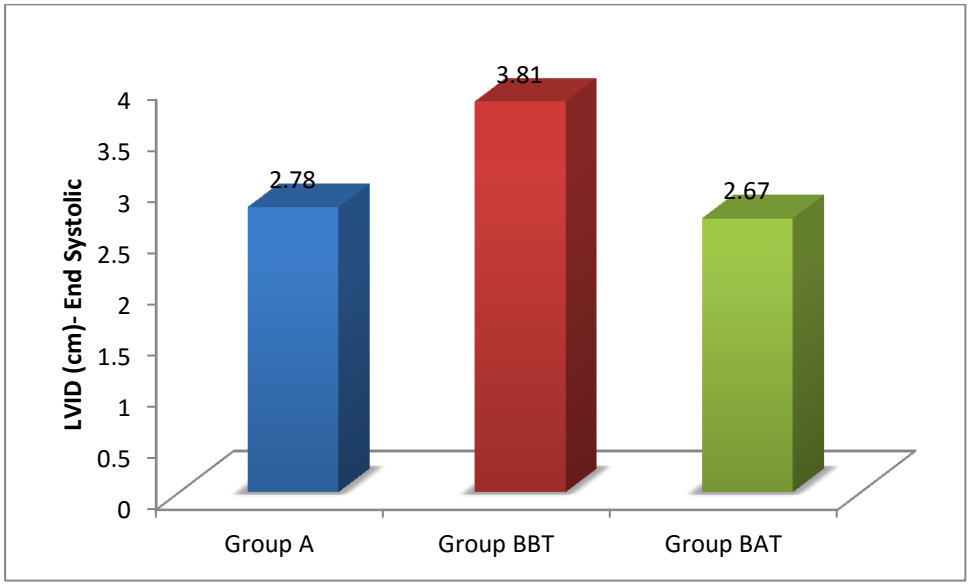
The mean value of LVID (End diastolic) between normal and hypothyroid individuals before treatment as well as after therapy was very highly significant.

Graph3. Comparison of ECHO parameters among study groups

3a. Ejection fraction (%)



3b. LVID (cm)- End Systolic



3c. LVID (cm) – End Diastolic

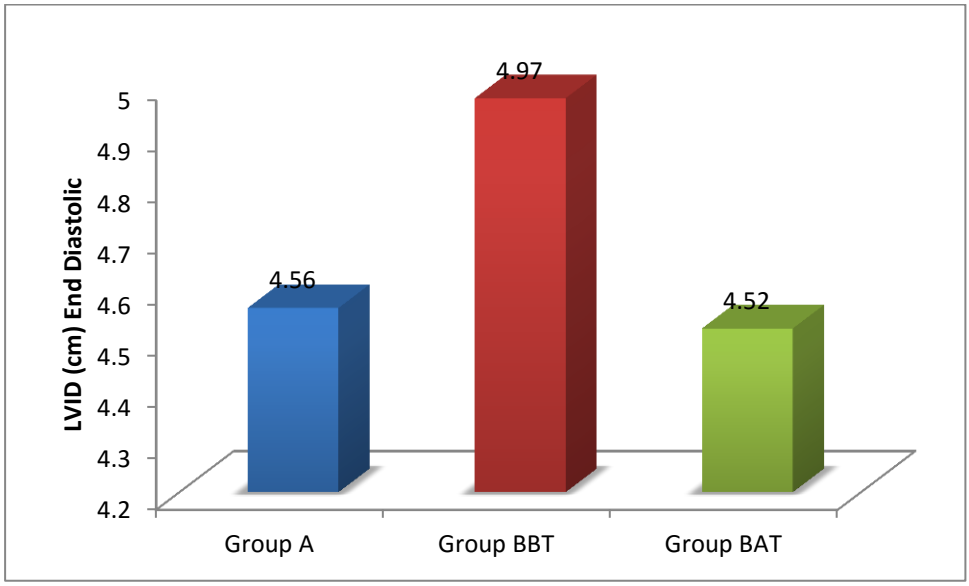


TABLE-V **Comparison of Time Domain measures of HRV**

Parameters	Study groups	N	Mean ± SD	P value
Mean Heart Rate/min	Group A	30	79.20 ± 5.96	0.000***
	Group B_{BT}	30	72.40 ± 6.64	
	Group B_{BT}	30	72.40 ± 6.64	0.002*
	Group B_{AT}	30	78.50 ± 8.90	
Mean RR interval in ms	Group A	30	807.17 ±124.49	0.0236*
	Group B_{BT}	30	869.03 ± 75.89	
	Group B_{BT}	30	869.03 ±75.89	0.794
	Group B_{AT}	30	800.43 ±94.09	
SDNN(ms)	Group A	30	44.37 ± 8.36	0.002*
	Group B_{BT}	30	37.53 ± 4.69	
	Group B_{BT}	30	37.53 ± 4.69	0.202
	Group B_{AT}	30	36.99 ± 4.74	
RMSSD(ms)	Group A	30	35.17 ± 6.86	0.545
	Group B_{BT}	30	33.76 ± 10.60	
	Group B_{BT}	30	33.76 ± 10.60	0.259
	Group B_{AT}	30	34.74 ± 8.67	

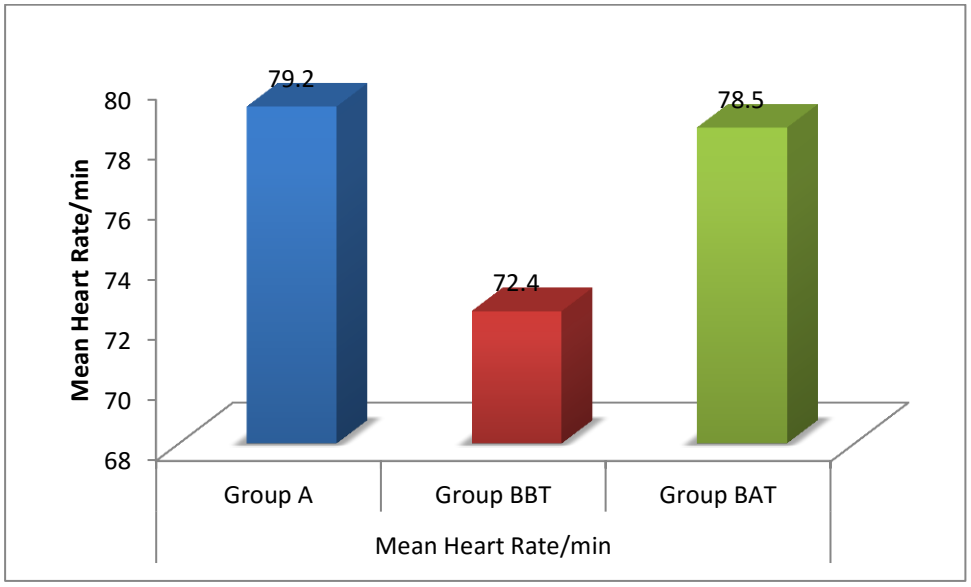
TABLE-V shows comparison of Time domain measures of Resting HRV among study groups. The mean value of Mean HR/min among the normal and hypothyroid individuals before therapy was highly significant. The mean value of Mean RR between normal and hypothyroid individuals before therapy was significant.

The mean value of SDNN between normal and hypothyroid individuals before therapy was significant.

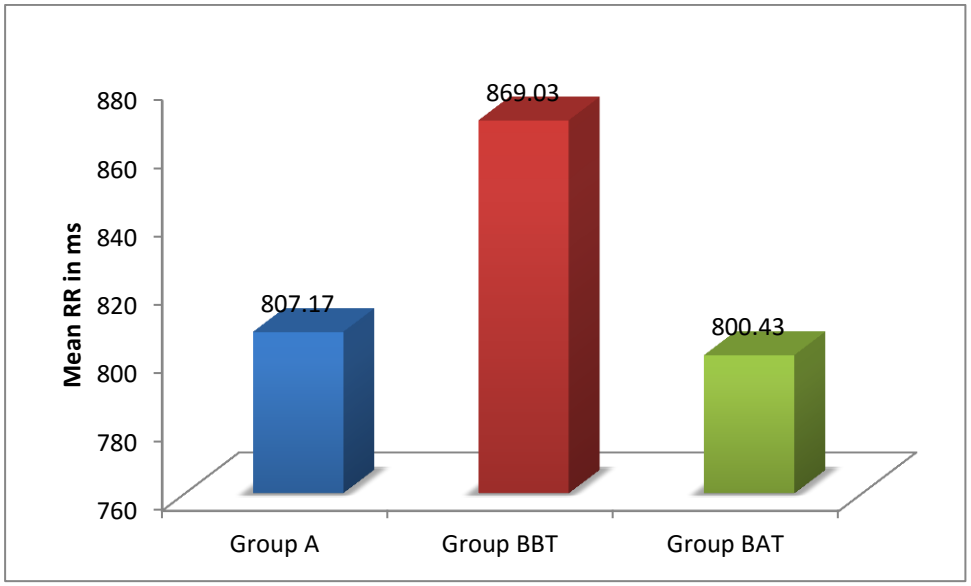
The mean RMSSD between the normal and hypothyroid individuals before therapy as well as after therapy was not significant.

Graph4. Comparison of HRV parameters- Time domain measures among study groups

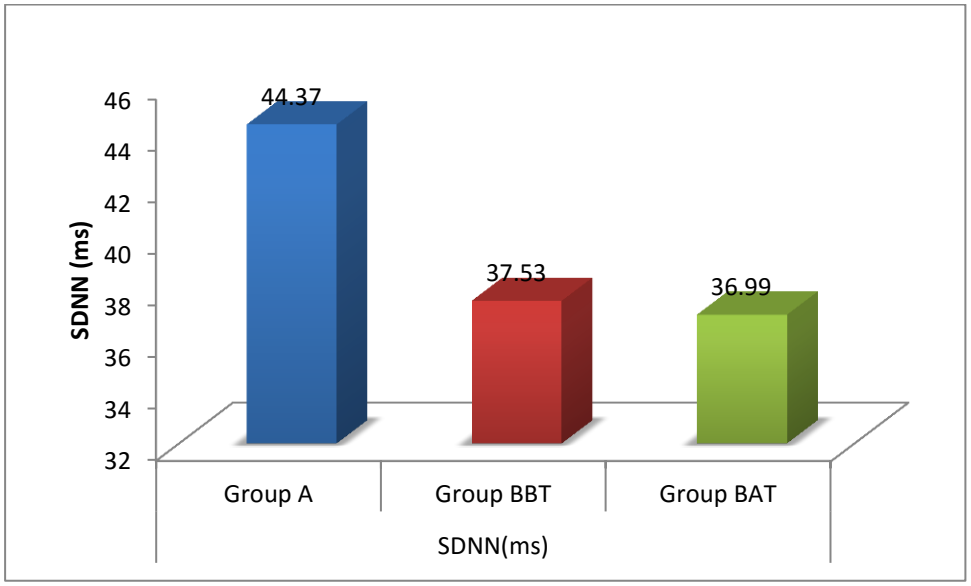
4a. Mean HR/min



4b. Mean RR(ms)



4c. SDNN(ms)



4d. RMSSD(ms)

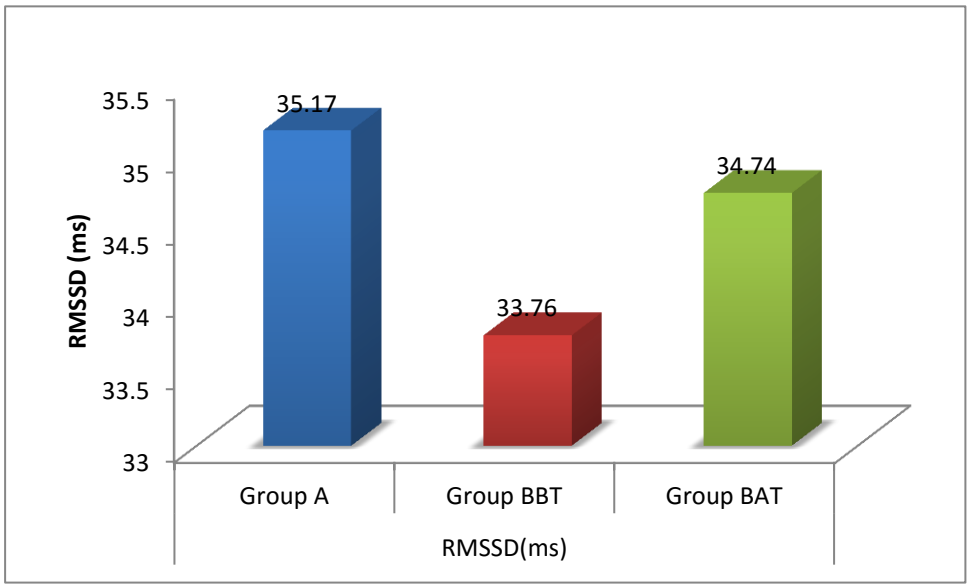


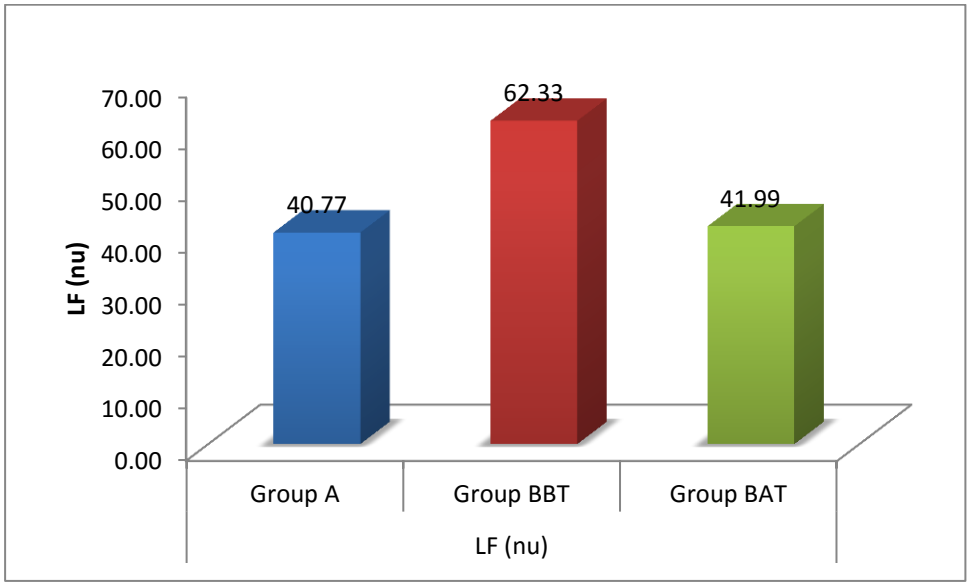
TABLE-VI **Comparison of Frequency Domain measures of HRV**

Parameters	Study groups	N	Mean ± SD	P value
LF (nu)	Group A	30	40.77 ± 5.63	0.000***
	Group B_{BT}	30	62.33 ± 9.02	
	Group B_{BT}	30	62.33 ± 9.02	0.000***
	Group B_{AT}	30	41.99 ± 5.95	
HF (nu)	Group A	30	59.23 ± 5.62	0.000***
	Group B_{BT}	30	37.07 ± 9.24	
	Group B_{BT}	30	37.07 ± 9.24	0.000***
	Group B_{AT}	30	58.01 ± 5.94	
LF HF Ratio	Group A	30	00.70 ± 0.16	0.000***
	Group B_{BT}	30	01.91 ± 0.98	
	Group B_{BT}	30	01.91 ± 0.98	0.000***
	Group B_{AT}	30	00.75 ± 0.18	
TOTAL Power (ms^2)	Group A	30	2546.23 ± 447.98	0.000***
	Group B_{BT}	30	1991.9 ± 545.26	
	Group B_{BT}	30	1991.9 ± 545.26	0.508
	Group B_{AT}	30	2084.7 ± 556.06	

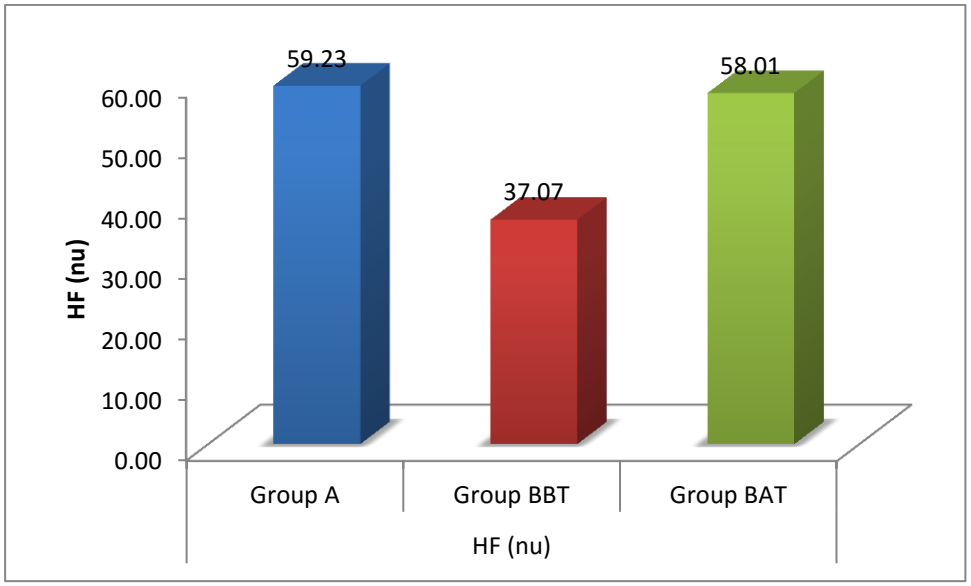
TABLE-VI shows comparison of Frequency domain measures of Resting HRV among study groups. The mean value of LF(nu), HF(nu), LF/HF ratio, Total power among the normal and hypothyroid individuals before and after therapy was very highly significant except the total power which did not showed significant difference after L-thyroxine therap.

Graph5. Comparison of HRV parameters- Frequency domain measures among study groups

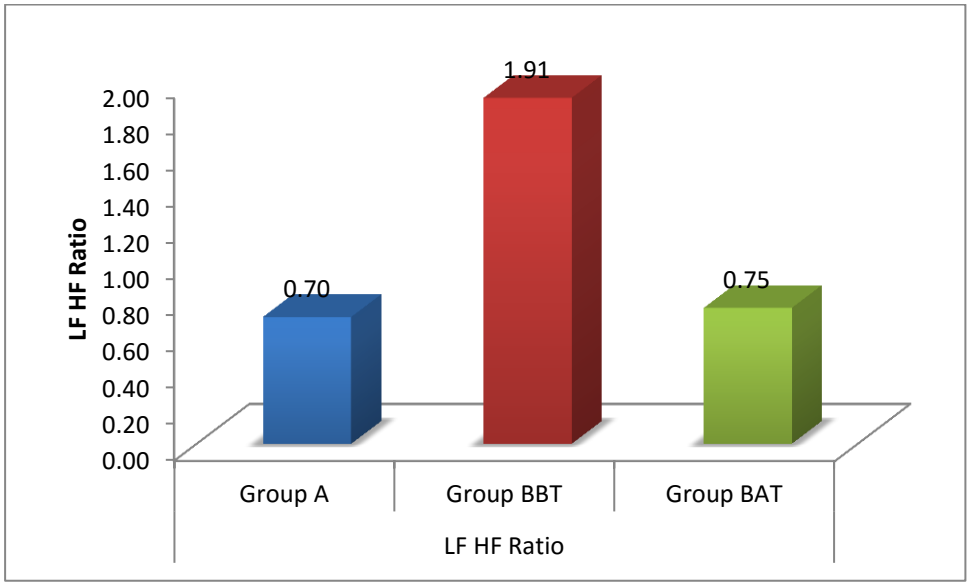
5a. LF (nu)



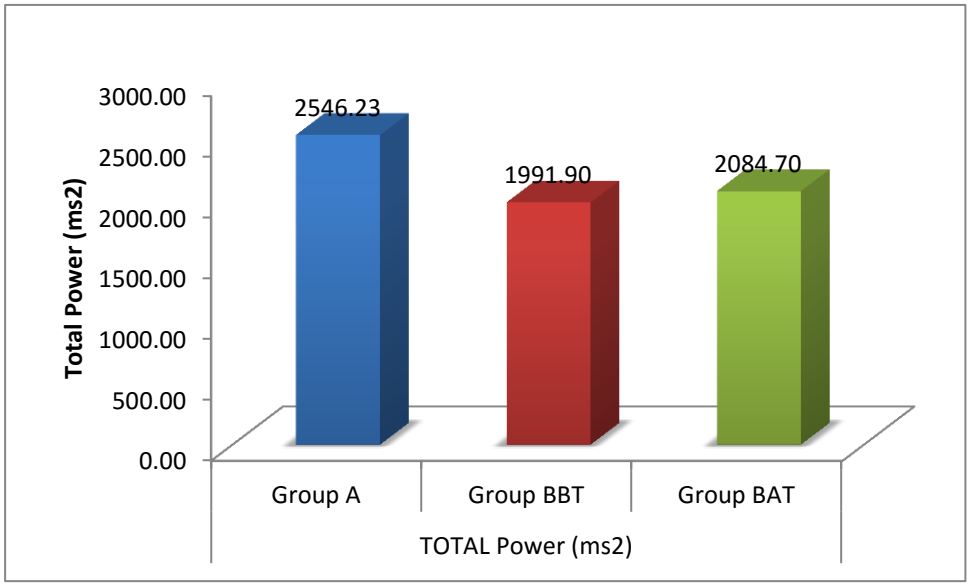
5b. HF (nu)



5c. LF/HF Ratio



5d. Total Power (ms^2)



DISCUSSION

DISCUSSION

Hypothyroidism has been found to be associated with increased cardiovascular morbidity and mortality. It affects cardiac structure, function, as well as cardiovascular hemodynamics **(Hills LD, Lange RA, Winniford MD, Page RL et al)[84].** Cardiac manifestations of hypothyroidism includes decreased heart rate, increased peripheral resistance, decreased cardiac output, decreased myocardial contractility, and disturbances in autonomic function.

This study was primarily to analyze the cardiac autonomic function in newly diagnosed hypothyroid female individuals by doing Heart Rate Variability Analysis and Echocardiography and the impact of L thyroxine over these parameters. Thirty newly diagnosed hypothyroid female subjects (with elevated serum TSH levels above 10mIU/ml) and thirty age matched normal controls were included in our study. After evaluating with HRV and ECHO the hypothyroid subjects were started on L thyroxine for a minimum of three months and serum TSH levels were measured after three months. After attaining euthyroid levels of serum TSH (0.5-5.5mIU/ml), the subjects were reassessed with HRV and ECHO. All the parameters were compared after therapy with L thyroxine.

The study groups were selected at an age group of 20 to 35 years to

alleviate the effects of ageing on autonomic dysfunction. The mean age of the control group was 27.50±3.37 and that of the Hypothyroid group was 27.23±3.77. The difference was not significant and so both the study groups are age matched. This was in accordance with the earlier studies where the selected study groups were at a similar age group.

Comparison of BMI in hypothyroidism

The body mass index (BMI) was calculated from the height and weight of the individuals by the formula weight (kg)/height in meters^2. The mean BMI of normal group was 25.97± 1.37 and the mean BMI of the hypothyroid individuals was 26.32±2.08, which was found to be statistically not significant. This was consistent with studies done by **(S. Karthick et al, G.K. Pal et al)[85].**

Resting Heart Rate and Blood Pressure in Hypothyroidism.

The mean Resting HR of the hypothyroid individuals before treatment was significantly lower than controls. An elevated level of sympathetic and decreased level of vagal modulation is expected in hypothyroidism. **(Cacciatori, Galetta, Frazoni F, Sahin et al)[86].** The mean HR of hypothyroid group was significantly lower than the normal control group. The possible reason for this could be sympathetic over activity. The mean Resting HR in hypothyroid subjects after treatment with L –thyroxine

showed statistically significant increase when compared with before treatment.

The Resting systolic blood pressure did not showed statistically significant difference among the normal and hypothyroid individuals before and after therapy. This was also found in study done by **(S. Karthick et al, G.K. Pal et al)**[85]. But there was a mild increase in diastolic blood pressure which was also found by **(Anjali Nadir Bhat et al, Leela Kalsotra et al)**[86].This could explain that there may be an increase in peripheral vascular resistance in hypothyroidism as suggested by**(Klein and Ojamaa et al 2000)**[37]**.** According to **(Fommei et al)**[39]**,** the decrease in thyroid hormone may cause the elevation of blood pressure levels and also triggering of sympathetic/adrenal system. This can be handled with replacement of thyroid hormone. The SBP and DBP can also be reduced with thyroxine therapies.

Serum TSH levels in hypothyroidism

In our study the serum TSH levels among the study groups were estimated by the ELISA method. A considerable amount of increase in mean serum levels of TSH is seen in people with hypothyroid than the normal group and this was found in the study done by **(S. Karthick et al, G.K. Pal et al)**[85]**.** After therapy with L-thyroxine the serum TSH levels was found to be

decreased to statistically significant levels which was also observed with study done by **(Vijayalakshmi et al, N. Vaney et al, S. V. Madhu et al)[14].**

Resting Heart Rate Variability.

Heart Rate Variability (HRV) analysis is one of the effective, non-invasive tool to assess the ANS function. There is an increased risk of adverse cardiac events in association with altered resting HRV. Analysis of the resting heart rate variability of a 5 minutes recording done using the HRV analysis software version1.1 among the study groups showed the following results.

Time domain measures:

Mean RR, Mean HR, SDNN, RMSSD were the variables taken for analysis as prescribed by the **Task force[74]**. Our findings showed decreased HRV in hypothyroid subjects. Decreased HRV is suggestive of either increased sympathetic tone or decreased parasympathetic tone. The Mean HR was significantly decreased in the hypothyroid individuals when compared to normal controls before treatment and it statistically improved after treatment with L thyroxine. The Mean RR among the study groups were measured which shows increased mean RR interval in the hypothyroid individuals before treatment when compared with normal controls. This shows relative bradycardia in the hypothyroid individuals as the mean RR

interval is inversely related with heart rate. Our findings were also observed in studies done by **(Galetta F et al, Franzoni F et al)**[87]. There was also minimal increase in mean RR intervals after treatment with L-thyroxine which was observed with earlier reporting of **(Inukai et al and Kahaly)**[45,46]**.**

In our study hypothyroid individuals showed decreased mean values of SDNN and RMSSD when compared with the normal controls. This was also observed with **(S. Karthick et al, G.K. Pal et al)**[85]**.** There was also mild increase in SDNN and RMSSD after treatment with L- thyroxine. Our findings were in accordance with **(Galetta F, Franzoni F, Fallahi P et al)**[87] who showed that the resting time domain measures were significantly lower in hypothyroid individuals and statistically improved after replacement with L-thyroxine. The SDNN, RMSSD were considered as sensitive indicators of parasympathetic function and thereby a low value indicates reduced vagal activity in hypothyroid individuals.

Frequency Domain Measures:

A significant variation in the frequency domain variables was observed among the study groups. The LF values which is an indicator of sympathetic tone was significantly higher in hypothyroid group when comparing with normal controls. The HF values in normalized units(nu) which is an indicator of parasympathetic tone was found to be significantly

lower in hypothyroid individuals before treatment when compared with normal controls This was found to be consistent with findings of **(Vittorio Cacciatori et al)[87].**

As the HF power is considered to be strictly under vagal activity, our findings suggests that there is decreased parasympathetic activity in hypothyroidism which were in line with earlier observations from **(Inukai et al)[16].** Moreover there was also restoration of the efferent vagal activity as evidenced by increase in HF power after treatment which indicates increased sympathetic to vagal nerve transportation to the heart in hypothyroid individuals.

A considerable increase in LF/HF ratio is seen in hypothyroid individuals before the treatment and the same is due to sympathovagal balance. This was also consistent with the reports from **(Galetta F, Franzoni F**, **Fallahi P, Tocchini L et al**)[86], who observed decreased HF, increased LF amplitude, increased LF/HF ratio in untreated hypothyroid individuals and significantly improved HF amplitude and comparable LF/HF ratios after treatment with replacement therapy with L thyroxine.

Our findings are consistent with prior studies of (**Cacciatori V, Sahin I, Turan N, Nielsen HV, Manhem P, Bramnert M, Bhat AN, Kalsotra L)[59,53,55,52]** who were observed significantly higher LF/HF ratio in hypothyroid individuals compared to control. Similar findings also found

by **(Matia Ahmed, Noorzahan Begum et al)**[58] in the hypothyroid subjects when compared with controls and treated hypothyroid patients.

It was found that reduction in vagal modulation and elevation in sympathetic happens with hypothyroidism. But key features of hypothyroidism are decreased HR. This is due to desensitization of receptor and also due to decreased cardiac chronotropic that refers to adrenergic stimulation although evidence of sympathetic over activity. This also explains the other features of hypothyroidism like increased peripheral resistance and increased diastolic blood pressure, peripheral vasoconstriction and cold intolerance in these individuals. The cardio vascular effects and changes in hypothyroidism is due to reduction in binding of catecholamine along with beta and alpha receptors of the cardiac myocytes **(Galetta et al)**[87]**.**

Total power in HRV indicates overall variability of RR interval and whole cardiac autonomic nervous actions and hormonal actions on the heart **(Task force).** In our study there is decreased total power prior to hypothyroid treatment when compared to normal control group which was in consistent with those observed by **(Caccitori, Galetta et al, Sahin Turan et al)**[52,53,55]**.** There is also improvement in the total power after treatment with L thyroxine which was observed in their studies.

Echocardiography in hypothyroidism

As echocardiography, which is also a non – invasive method plays an significant action in identifying the cardiac pathology and for the subsequent effect of the therapy **(Rodondi N, Bauer D.C., Cappola A.R., et al)[79].** In the present study we carried out an investigation of cardiac function in patients with newly diagnosed hypothyroid female individuals and followed up after attaining euthyroid state with L thyroxine replacement therapy. The ECHO parameter especially the ejection fraction and LVID (end systolic and diastolic together) were compared with those of normal controls after therapy.

Our present study showed very highly significant decrease in ejection fraction in hypothyroid individuals when compared with normal controls. It was also improved after treatment with L thyroxine almost to normal range with very high significant pvalue of 0.000. This was in accordance with previous studies done by **(Rawat B, Satyal A, Monzani F, Bello VD, Caraccio N et al)[88].**

The left ventricular internal diameter together with end systolic and end diastolic dimensions are taken in cms. There were stastically significant differences in the parameters among the study groups that is between normal and hypothyroid individuals before and after therapy with L

thyroxine treatment. Our findings are consistent with those observed by **(Rawat B, Satya et al)[88].**

In the present study, based on the discussion, we observed a substantial fall in total power in hypothyroid patients in relation to normal controls from the spectral frequency domain analysis for HRV. This reduction in total power reflects the reduced vagal modulation in the individuals. **(Alberto M et al, Task force of European Society**)[89]. This finding contradicts with those observed by **Xing et al[54]**. According to Xing, high HF values in hypothyroid individuals have elevated vagal tone with autonomic imbalance. **Xing et al[54]** found HF more than the LF segment. But in our study, it showed decreased HF than LF, which denotes reduced vagal activity. We observed that the difference in observation is may be due to different sample size.

It is also observed that the contribution of HF is higher than the LF - VF components with the ratio of two third to one third respectively. This explains the reduction in vagal activities due to decreased HF power in the hypothyroid individuals may result in reduced total power.

The normal cardiovascular health of the individuals is determined by vagal activity on the heart. Hence vagal innervation has got greater influence on the control of heart rate, BP and cardia output. Our study shows increased LF power in the hypothyroid individuals which was also

reported by **Cacciatori et al**[52]. The most probable reason for that may be due to increased sympathetic activity on the heart..

Thus the results of the resting heart rate variability in hypothyroid individuals differed noticeably from the results of those obtained in the normal individuals. This represents a sympathovagal imbalance in hypothyroid individuals with sympathetic over activity and altered functional status of the heart as shown by ECHO parameters. Both these altered functional status and sympathovagal imbalance can be improved by early replacement therapy with L thyroxine. This could prevent the cardiovascular complications of hypothyroidism like cardiac arrhythmias in these patients.

LIMITATIONS OF THE STUDY

Our study was done in a smaller sample size. It is necessary to substantiate our findings and apply it to a general population studies using larger sample size. All the women were selected in the 20-35 years of age. So in order to generalize the findings, larger studies including wide age groups should be included.

This study was conducted on newly diagnosed hypothyroid female individuals who were not on any treatment in whom the duration of the

disease was not known. So we could not find any correlation between the duration of the disease and autonomic dysfunction.

The study had a drawback, as it evaluated only the resting autonomic activity and not the response of the autonomic nervous system to various external stimuli or lab stressors.

The mechanisms suggested for autonomic dysfunction in hypothyroidism are an elevated circulating nor epinephrine levels with reduced sensitivity of the receptors to the stimuli. Hence our observations need to be substantiated by measurements of the catecholamine nor epinephrine levels which will not be feasible here.

From this study, ultimately we understood that autonomic imbalance could be a risk factor for cardiovascular morbidity among hypothyroid individuals and it could be prevented by adequate replacement therapy with L thyroxine.

CONCLUSION

CONCLUSION

The cardiovascular autonomic nervous system activity and functional status of the heart were evaluated in newly diagnosed hypothyroid female individuals using Resting Heart rate variability analysis and Echocardiographic study before starting on treatment. Further the subjects are followed with 3 months of replacement therapy with L thyroxine. After obtaining euthyroid state the subjects were reevaluated again and the observed parameters were compared.

This study concludes an autonomic imbalance as evidenced by decrease in SDNN, RMSSD which were indicators of parasympathetic activity in hypothyroid individuals before treatment and increase in LF power (nu) showing the sympathetic activity. The ratio between LF and HF was increased in hypothyroid individuals before treatment which showed sympathetic dominance of autonomic nervous system activity. The total power is also decreased in hypothyroid individuals when compared to normal controls.

When analyzing the Echocardiographic parameters there is decrease in ejection fraction and there were differences in LVID (end systolic, end diastolic). This shows altered structural and functional status of the heart in hypothyroidism.

All these parameters showed significant improvement after replacement therapy with L thyroxine.

SUMMARY

SUMMARY

Hypothyroidism is a common endocrine disorder with female preponderance. It is known to be associated with autonomic dysfunction and cardiovascular risks like hypertension, atherosclerosis, and arrhythmias. We aimed to evaluate the autonomic system activity by using Resting heart rate variability analysis and echocardiography in newly diagnosed (clinically as well as biochemically with elevated serum TSH levels) female hypothyroid individuals.

Thirty female hypothyroid individuals in the age group of 20-35 years with age matched healthy females were taken as controls. Both of them subjected to Resting HRV and ECHO. The cases were followed up after 3 months of replacement therapy with L thyroxine. Reassessedwith HRV and ECHO after attaining euthyroid state.

Analysis of the data obtained from the study, showed that hypothyroidism is associated with autonomic dysfunction in the form of increased sympathetic activity and decreased parasympathetic activity compared to their controls with altered functional status of the heart as evidenced by echocardiography. This could be improved by replacement therapy with L thyroxine as which was shown by reassessing the cases after treatment.

.All these observation suggests that sympathetic over activity with decreased parasympathetic activity in hypothyroidism might be responsible for the pathogenesis of cardiovascular features associated with hypothyroidism.

And improvement in HRV and echo parameters after treatment with L thyroxine shows that the cardiovascular autonomic function can be maintained with adequate replacement therapy in hypothyroidism and potential cardiovascular risks associated with it can be prevented by regular follow up of these individuals with these noninvasive tests.

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89.European Journal of Endocrinology (2000) 143 327±333 ISSN 0804-4643 CLINICAL STUDY Power spectral analysis of heart rate in hypothyroidism

Vittorio Cacciatori, Maria Luisa Gemma, Federico Bellavere, Roberto Castello, Maria Emilia De Gregori, Giacomo Zoppini, Karl Thomaseth1, Paolo Moghetti and Michele Muggeo

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ANNEXURES

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No. 044 25305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To
Dr. G.Saraswathi,
Postgraduate M.D.(Physiology),
Madras Medical College,
Chennai – 600 003.

Dear Dr. G.Saraswathi,

The Institutional Ethics Committee has considered your request and approved your study titled **"Heart rate variability analysis and Echocardiographic study in normal and hypothyroid (female) patients before and after treatment" No.12092014.**

The following members of Ethics Committee were present in the meeting held on 02.09.2014 conducted at Madras Medical College, Chennai-3.

1.	Dr.C.Rajendran, M.D.,	: Chairperson
2.	Dr.R.Vimala, M.D., Dean, MMC, Ch-3	: Deputy Chairperson
3.	Prof.D.Kalaiselvi, M.D., Vice-Principal, MMC, Ch-3	: Member Secretary
4.	Prof.R.Nandhini, M.D., Inst.of Pharmacology, MMC	: Member
5.	Dr.G.Muralidharan, Director Incharge, Inst.of Surgery	: Member
6.	Prof.K.Ramadevi, Director i/c, Inst.of Biochemistry, MMC	: Member
7.	Prof.Saraswathy, M.D., Director, Pathology, MMC, Ch-3	: Member
8.	Prof.Tito, M.D., Director i/c, Inst.of Internal Medicine, MMC	: Member
9.	Thiru S.Rameshkumar, Administrative Officer	: Lay Person
10.	Thiru S.Govindasamy, B.A., B.L.,	: Lawyer
11.	Tmt.Arnold Saulina, M.A., MSW.,	: Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

Member Secretary, Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

INFORMED CONSENT FORM

Title of the study: "Heart rate variability analysis and Echocardiographic study" in normal and Hypothyroid(female) patients before and after treatment.

Name of the Participant:

Name of the Principal Investigator: Dr.G.Saraswathi

Name of the Institution:

Institute of Physiology and Experimental Medicine,
Madras Medical College and Govt. General Hospital,
Chennai - 3

Documentation of the informed consent

I _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I am over 18 years of age and, exercising my free power of choice, hereby give my consent to be included as a participant. "Heart rate variability analysis and Echocardiographic study" in normal and Hypothyroid patients before and after treatment

1. I have read and understood this consent form and the information provided to me.
2. I have had the consent document explained to me.
3. I have been explained about the nature of the study.
4. I have been explained about my rights and responsibilities by the investigator.
5. I have been informed the investigator of all the treatments I am taking or have taken in the past _____ months including any native (alternative) treatment.
6. I have been advised about the risks associated with my participation in this study.
7. I agree to cooperate with the investigator and I will inform him/her immediately if I suffer unusual symptoms.
8. I have not participated in any research study within the past _____month(s).
9. I am aware of the fact that I can opt out of the study at any time without having to give any reason and this will not affect my future treatment in this hospital.
10. I am also aware that the investigator may terminate my participation in the study at any time, for any reason, without my consent.

12. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.
13. I have understand that my identity will be kept confidential if my data are publicly presented.
14. I have had my questions answered to my satisfaction.
15. I have decided to be in the research study.
I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

For adult participants:
Name and signature / thumb impression of the participant (or legal representative if participant incompetent)

Name _____ Signature_____

Date_____

Name and Signature of impartial witness (required for illiterate patients):

Name _____ Signature_____

Date_____

Address and contact number of the impartial witness:

Name and Signature of the investigator or his representative obtaining consent:

Name _____ Signature_____

Date_____

ஆராய்ச்சி ஒப்புதல் படிவம்

ஆராய்ச்சி தலைப்பு:

ஆராய்ச்சியாளர் பெயர்: மரு. சரஸ்வதி

ஆராய்ச்சி நடக்கும் இடம்: சென்னை மருத்துவக் கல்லூரி

பெயர்:

வயது:

பாலினம்: ஆண்/ பெண்

முகவரி:

பங்கு பெறுபவர் அடையாள எண்:

இந்த ஆராய்ச்சியின் விவரங்களும் அதன் நோக்கமும் முழுமையாக எனக்கு தெளிவாக விளக்கப்பட்டது.

எனக்கு விளக்கப்பட்ட விஷயங்களை புரிந்து கொண்டு நான் எனது சம்மதத்தை தெரிவிக்கிறேன்.

இந்த ஆராய்ச்சியில் யாருடைய நிர்பந்தமுமின்றி கொத்த விரும்புதலின் பேரில் சம்மதிக்கிறேன்.

இந்த ஆராய்ச்சியில் இருந்து நான் எந்த நேரமும் பின் வாங்கலாம் என்றும் அதனால் எந்த பாதிப்பும் ஏற்படாது என்பதையும் புரிந்து கொண்டேன்.

தைராய்டு கரப்பி பற்றாக்குறையினால் அட்டானாமிக் நரம்பு மண்டலத்தில் ஏற்படும் மாற்றங்களை இருதய துடிப்பு வேறுபடுதல் மற்றும் இருதய ஸ்கேன் மூலமாக கண்டறிந்து, சிகிச்சைக்குப் பின் ஏற்படும் மாற்றங்களை ஒப்பிடுதல் பற்றிய இந்த ஆராய்ச்சியின் விவரங்கள் கொண்ட தகவல்களை பெற்றுக்கொண்டேன்.

நான் இருதய துடிப்பு வேறுபடுதல் மற்றும் இருதய ஸ்கேன் செய்து கொள்ளவும் சம்மதிக்கிறேன்.

நான் என்னுடைய சுய நினைவுடன் மற்றும் முழு சம்மதத்துடன் இந்த ஆராய்ச்சிக்கு என்னை பரிசோதிக்க சம்மதிக்கிறேன்.

பங்கேற்பாளர் கையொப்பம்

நாள்:

இடம்:

PROFORMA

1. Name :
2. Age:
3. Sex:
4. Address :
5. Occupation:
6. Symptoms suggestive of hypothyroidism:
7. History of any drug intake:
8. Past medical or surgical history:
   - Diabetes mellitus
   - Hypertension
   - Ischemic heart disease
   - Bronchial asthma
   - Malignancy
   - Surgery on Thyroid gland
9. Family history:
10. Personal history:
11. Clinical examination:

General examination

Pulse rate :

Blood pressure :

Systemic examination :

Cardiovascular system :

Respiratory system :

Gastrointestinal system :

Central nervous system :

Investigations :

1. Hemoglobin estimation :
2. Thyroid profile :
3. Heart rate variability analysis :
4. 2D Echocardiography :
5. Electrocardiography :

MASTER CHART CONTROL

Control No.	AGE	BMI	HR/min resting	SBP /resting	DBP /resting	TSH	EF%	LVID (cm)		Mean HR /min	Mean RR /min	SDNN	RMSSD	LF	HF	LF/HF	Total power
								ES	ED								
1	26	20.5	76	120	72	0.8	60	2.8	4.3	86	678	54.7	41.3	42.5	57.5	0.739	2564
2	30	24.4	74	118	70	1.2	58	2.4	4.1	84	890	36.4	40.2	34.6	65.4	0.529	2132
3	28	23.8	80	122	68	3.4	56	2.8	4.5	85	810	38.1	28.2	46.9	53.1	0.883	1318
4	27	22.4	82	126	78	2.8	55	2.7	4.3	86	810	52.6	41.1	32.6	67.4	0.484	2765
5	32	23.8	79	124	74	3.1	60	2.5	4.7	84	980	62.7	55.7	38.6	61.4	0.629	2786
6	29	24.7	75	100	72	2.2	65	2.4	4.2	86	576	37.6	41.1	27.8	72.2	0.385	1286
7	27	24.2	78	110	76	2.5	58	2.7	4.3	85	458	36.5	16.3	33.1	66.9	0.495	2546
8	28	23.6	76	116	76	3.5	60	2.8	4.7	79	593	46.9	30.6	43.7	56.3	0.776	2785
9	24	24.2	74	114	74	4.2	62	2.6	4.8	78	759	48.1	43	35.4	64.6	0.548	2784
10	22	21.8	76	120	76	4.5	56	2.5	4.6	82	859	57.4	25.3	43.8	56.2	0.779	2493
11	30	24.4	80	116	80	3.4	58	2.9	4.5	80	987	54.9	27.6	38.8	61.2	0.634	2962
12	28	24.5	76	122	74	2.8	60	2.8	4.8	79	890	44.6	34.8	31.8	68.2	0.466	2341
13	27	22.8	74	118	72	2.7	62	2.9	4.4	80	810	46.7	32.7	34.2	65.8	0.52	2808
14	29	24.1	80	116	76	3.8	58	2.7	4.8	81	880	65.9	33.7	45.3	54.7	0.828	2370
15	24	23.3	78	110	70	3.2	59	2.9	4.9	76	859	34.9	34.6	42.8	57.2	0.748	2786
16	25	21.7	82	112	76	3.8	58	3.1	4.7	84	865	36.4	38.9	43.7	56.3	0.776	3432
17	27	24.4	84	114	72	4.7	57	2.6	4.5	82	759	46.7	36.7	42.6	57.4	0.742	2439
18	30	23.9	80	118	74	4.5	62	2.8	4.8	69	887	45.8	35.9	44.2	55.8	0.792	2345
19	32	22.9	76	120	78	2.2	65	2.9	4.9	70	890	36.6	32.6	48.3	51.7	0.934	2349
20	34	24.2	76	108	78	2.7	58	3.1	4.2	78	960	49.8	38.2	39.4	60.6	0.65	2916
21	31	25	78	110	76	3.5	56	3	4.7	79	859	43.9	34.9	46.2	53.8	0.859	3108
22	28	21.6	82	122	74	5.5	60	2.9	4.8	77	810	39.7	35.4	46.9	53.1	0.883	2547
23	26	24.9	76	114	72	4.8	62	3.1	4.7	73	730	45.4	39.7	49.4	50.6	0.976	2980
24	25	23.8	75	116	74	4.1	64	2.8	4.8	75	812	37.9	29.8	42.1	57.9	0.727	2312
25	27	22.5	74	118	70	3.4	63	2.6	4.6	72	676	36.8	37.9	40.4	59.6	0.678	2438
26	23	25.1	76	120	80	3.8	59	2.9	4.9	74	823	37.9	36.1	47.2	52.8	0.894	2132
27	22	24.4	75	122	82	3.2	55	3	4.8	73	1008	39.9	27.5	37.7	62.3	0.605	2643
28	35	24.2	78	126	74	2.6	65	2.9	4.3	86	786	40.8	37.9	44.9	55.1	0.815	2490
29	22	24.1	80	118	76	2.8	58	2.8	4.2	79	692	38.2	33.5	34.5	65.5	0.527	2983
30	27	23.6	82	120	80	3.5	62	2.5	4.1	94	819	37.4	33.8	43.8	56.2	0.779	2547

MASTER CHART BEFORE TREATMENT

Case no.	AGE	BMI	HR/min resting	SBP /resting	DBP /resting	TSH	EF%	LVID (cm)		Mean HR /min	Mean RR /min	SDNN	RMSSD	LF	HF	LF/HF	Total power
								ES	ED								
1	23	24.4	70	110	70	7.5	53	4	5.4	76	692	46.5	34.3	59.5	40.5	1.469	1324
2	27	25.3	78	114	76	10.2	50	3.7	5.5	70	798	35.2	30.6	54.9	45.1	1.217	2312
3	24	26.1	70	120	78	8.7	52	3.8	4.9	72	785	45.1	37.8	67.9	32.1	2.115	1345
4	30	25.1	74	116	76	15.6	54	3.6	4.8	72	692	36.9	24.8	59.6	40.4	1.475	2113
5	29	24.8	72	110	80	20.5	52	3.4	4.6	66	798	45.9	38.2	50.6	49.4	1.024	2145
6	28	27.3	80	112	70	18.3	50	4	5.2	80	854	23.8	16.3	62.1	37.9	1.638	1484
7	26	26.5	79	120	76	27.9	47	3.9	5.1	82	692	36.4	40.2	75.6	24.4	3.098	2134
8	21	28.1	72	118	76	32.6	49	3.8	5	80	913	38.5	33.6	58.7	41.3	1.421	2310
9	22	27.9	80	114	78	15.3	43	3.7	5.6	78	902	26.9	16.3	62.4	37.6	1.659	1484
10	27	25.7	70	120	82	22.6	47	3.9	4.9	71	896	34.7	29.6	79.4	20.6	3.854	2765
11	23	26.2	69	114	76	28.4	46	3.5	4.8	60	884	39.8	47.3	57.3	22.7	2.524	1346
12	24	27.4	70	120	74	39.3	48	3.4	4.9	70	913	35.2	30.6	54.9	45.1	1.217	2295
13	27	25.3	72	130	76	46.5	50	3.8	4.7	72	892	36.9	24.8	59.6	42.3	1.408	1984
14	32	24.3	72	132	78	29.8	47	3.7	4.8	68	878	35.6	30.9	82.3	17.7	4.649	2312
15	25	24.1	69	126	74	32.6	45	3.6	4.5	71	923	34.1	29.6	79.4	20.6	3.854	2901
16	29	23.8	67	128	90	34.9	51	3.9	4.9	67	897	38.2	58.5	56.8	43.2	1.314	2564
17	28	25.7	70	120	84	24.8	49	3.7	5	80	904	39.6	16.3	62.1	37.9	1.638	1453
18	27	24.9	60	116	82	17.5	54	3.6	5.3	60	967	39.8	47.3	57.3	42.7	1.341	1458
19	23	25.7	72	126	82	40.6	49	3.6	5.7	70	956	35.2	30.6	54.9	45.1	1.217	2342
20	28	26.2	68	124	76	37.9	50	3.8	5.1	68	913	35.8	30.9	60.6	39.4	1.538	1129
21	29	25.8	66	120	74	44.3	52	3.7	5	71	908	34.7	29.6	79.4	20.6	3.854	2345
22	30	27.3	68	122	76	18.6	51	3.5	4.9	72	920	36.9	24.8	59.6	40.4	1.475	1456
23	33	28.6	82	116	74	23.5	47	4	4.7	82	964	38.5	33.6	58.1	41.9	1.386	2350
24	32	27.2	69	118	78	43.4	48	3.9	4.8	74	921	37.8	36.1	52.8	47.2	1.104	1113
25	29	28.1	80	120	80	29.5	53	4.1	4.7	80	879	40.7	36.3	70.1	29.9	2.344	2907
26	34	23.6	70	134	82	27.9	54	4.3	5.5	73	856	36.2	24.8	59.6	40.4	1.475	1328
27	21	27.6	60	116	80	42.1	55	4.2	4.6	56	893	39.7	47.5	57.7	42.3	1.364	2005
28	24	25.8	84	110	76	53.5	48	4.1	4.8	76	879	37.9	35.1	50.2	49.8	1.008	2657
29	27	24.9	80	116	78	49.8	47	4	4.9	82	913	39.6	37.3	56.1	43.9	1.277	2031
30	35	25.5	76	132	84	38.7	49	4.1	4.7	73	789	43.7	59.3	70.3	29.7	2.367	2365

MASTER CHART AFTER TREATMENT																	
Case no.	AGE	BMI	HR/min resting	SBP /resting	DBP /resting	TSH	EF%	LVID (cm)		Mean HR /min	Mean RR /min	SDNN	RMSSD	LF	HF	LF/HF	Total power
								ES	ED								
1	27	25.3	78	116	74	4.2	55	2.3	4	84	714	46.9	34.2	36.2	63.7	0.567	1087
2	24	26.1	70	120	80	4.8	58	2.3	4.2	81	741	35.8	30.7	49.4	50.6	0.976	2901
3	30	25.1	77	114	78	5.3	62	2.4	4.1	79	762	45.2	37.9	31.1	68.9	0.451	2691
4	29	34.8	70	112	76	5.5	63	2.8	4.5	69	866	36.8	25.9	43.8	56.2	0.779	2126
5	28	27.3	84	110	68	4.6	59	2.7	4.3	87	704	45.7	39.1	41.1	58.9	0.697	1768
6	26	26.5	92	124	76	4.9	54	2.3	4.7	94	632	23.8	17	46.1	53.9	0.855	2130
7	21	28.1	90	118	72	3.7	60	2.6	4.7	93	792	36.1	41.5	41.8	58.2	0.718	1298
8	22	27.9	88	116	78	3.1	52	2.8	4.8	90	823	33.2	33.9	43.9	56.1	0.782	2902
9	27	25.7	78	120	76	5.4	55	2.6	4.6	78	764	26.8	19.8	46.5	53.5	0.869	1797
10	23	26.2	60	114	76	1.8	53	2.5	4.5	63	986	34.6	32.8	45.9	54.1	0.848	1456
11	24	27.4	72	120	72	2.2	51	2.9	4.5	74	867	38.7	47.1	37.5	62.5	0.6	1893
12	27	25.3	75	132	70	3.4	58	2.8	4.4	75	852	35.1	32.8	42.5	57.5	0.739	1329
13	32	24.3	74	130	74	3.7	56	2.4	4.1	76	782	36.9	24.8	38.1	61.9	0.615	2873
14	25	24.1	75	128	76	4.7	59	2.8	4.5	76	782	35.7	34.8	37.9	62.1	0.61	2876
15	29	23.8	72	126	76	4.7	59	2.7	4.3	73	813	34.2	35.6	42.1	57.9	0.727	1241
16	28	25.7	84	118	72	4.2	58	2.3	4.7	86	764	38.6	37.3	49.8	50.2	0.992	1515
17	27	24.9	65	114	74	4.3	65	2.4	4.2	68	947	39.7	19.5	32.4	67.6	0.479	1863
18	23	25.4	74	124	70	5.2	57	2.7	4.3	73	816	39.6	40.9	42.2	57.8	0.73	2598
19	28	26.2	78	122	78	5	58	2.8	4.7	75	853	35.5	32.8	45.1	54.9	0.821	2735
20	29	25.8	76	118	72	4.9	63	2.6	4.8	78	776	35.9	34.7	48.8	51.2	0.953	1609
21	30	27.3	78	120	70	5.5	60	2.5	4.6	75	852	34.8	29.8	42.4	57.6	0.736	2342
22	33	28.6	88	112	74	3.8	59	2.9	4.5	87	694	36.8	30.6	34.7	65.3	0.531	2676
23	32	27.2	75	114	76	3.5	55	2.8	4.8	76	786	39.1	33.7	49.8	50.2	0.992	1986
24	29	28.1	92	118	72	4.9	63	2.9	4.4	78	767	37.9	39.6	48.8	51.2	0.953	1609
25	34	23.6	76	132	80	3.4	63	3.1	4.8	94	654	41.8	45.7	46.2	53.8	1.17	2166
26	21	27.6	63	110	82	2.7	65	2.8	4.9	59	1008	37.3	27.5	42.3	57.7	0.733	1995
27	24	25.8	82	100	74	4.9	59	3	4.7	80	747	40.1	47.9	42.6	57.4	0.742	2264
28	27	24.9	88	114	76	3.8	57	2.6	4.5	89	698	33.4	40.2	24.2	75.8	0.319	2315
29	35	25.5	76	130	80	3.9	55	2.9	4.8	78	776	40.1	37.3	43.8	56.2	0.779	1798
30	33	25.3	80	124	78	4.8	60	3.1	4.9	62	995	33.7	56.8	42.7	57.3	0.745	2702